HIV Type 1 Infection of Plasmacytoid and Myeloid Dendritic Cells Is Restricted by High Levels of SAMHD1 and Cannot be Counteracted by Vpx

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Abstract

Dendritic cells are professional antigen-presenting cells of the immune system and are major producers of type-I interferon. Their role in HIV-1 infection is not well understood. They express CD4 and CCR5 yet appear to be resistant to infection. In culture, infection of the cells with HIV-1 is inhibited by the host cell restriction factor SAMHD1. Lentiviruses such as HIV-2/SIVmac counteract the restriction by encoding Vpx, a virion-packaged accessory protein that induces the proteasomal degradation of SAMHD1. In this study we investigated SAMHD1-mediated restriction in the two major dendritic cell subsets: plasmacytoid dendritic cells (pDC) and myeloid dendritic cells (mDC). The cells were highly resistant to HIV-1 and expressed high levels of SAMHD1. SAMHD1 amino acid residue T592, a target of CDK1 phosphorylation, was unphosphorylated, corresponding to the antiviral form of the enzyme. The resistance to infection was not counteracted by Vpx and SAMHD1 was not degraded in these cells. Treatment of pDCs with a cocktail of antibodies that blocked type-I interferon signaling partially restored the ability of Vpx to induce SAMHD1 degradation and caused the cells to become partially permissive to infection. pDCs and mDCs responded to HIV-1 virions by inducing an innate immune response but did not appear to sense newly produced Gag protein. The findings suggest that *in vivo*, dendritic cells serve as sentinels to alert the immune system to the virus but do not themselves become infected by virtue of high levels of SAMHD1.

Introduction

DENDRITIC CELLS (DC) are professional antigen-presenting cells that play a central role in adaptive and innate immune responses. They are divided into two major subtypes, myeloid (mDC) and plasmacytoid (pDC). mDCs recognize diverse pathogens, express an array of Toll-like receptors (TLR), and produce cytokines that influence Th1, Th2, Th17, and regulatory T cell (Treg) development. CD14⁺ monocytes can be differentiated in culture with granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 to yield monocyte-derived dendritic cells (MDDC), a cell type that has been used to model primary mDCs.^{1,2} pDCs are characterized by their plasmacytoid morphology and ability to secrete high levels of type-I interferon (IFN). They respond to a more limited set of pathogens and express TLR7 and TLR9, which recognize single strand RNA and unmethylated CpG

DNA, respectively. They do not secrete Th1 skewing cytokines, such as IL-12, but produce high levels of type-I IFN,³ conferring resistance to productive infection by many viruses. Unlike RNA viruses such as influenza, HIV-1 does not activate mDCs or pDCs to become antigen-presenting cells,^{4,5} which may contribute to inadequate adaptive anti-HIV-1 immune response development.

pDCs also contribute to chronic inflammation in HIV-1 infection by producing proinflammatory cytokines and chemokines^{6–8} and may suppress the immune response by producing indoleamine (2,3)-dioxygenase (IDO)^{9,10} which induces Treg differentiation. pDCs likely play a role in the early stages of infection by recruiting CCR5⁺ CD4⁺ T cells to mucosal sites of transmission¹¹ and by inducing the activation and apoptosis of CD4⁺ T cells through the production of type-I IFN.¹² While mDCs and pDCs express CD4 and CCR5 and can bind and internalize HIV-1, they are resistant to infection *in vitro*.^{13–15}

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In MDDCs, the block to infection is mediated by the host restriction factor sterile alpha motif and HD domain 1 protein (SAMHD1),^{16,17} a phosphohydrolase that dephosphorylates the pool of deoxynucleotide triphosphates (dNTP) in nondividing cells, depleting the intracellular dNTP pool to a level below that required for HIV-1 reverse transcription.^{18–20} The block extends to retroviruses in general^{21,22} and to other DNA-dependent viruses.²³ SAMHD1 also blocks the infection of resting T cells but not activated CD4⁺ T cells^{24,25} or other cycling cells. The antiviral activity of SAMHD1 is regulated by CDK1 phosphorylation at amino acid T592, and in cycling cells most of the SAMHD1 is phosphorylated, limiting its antiviral activity.^{26,27}

Lentiviruses including SIV_{mac}, SIV_{agm}, SIV_{sm}, and HIV-2 counteract SAMHD1 by encoding the accessory protein, Vpx (or in SIV_{agm}, Vpr),^{28–30} a small nuclear protein that is packaged into virions through an interaction with the Gag precursor polyprotein. Upon entry into the cell, Vpx is thought to transit to the nucleus where it associates with SAMHD1 and recruits a CRL4 E3 ubiquitin ligase complex consisting of DNA damage binding protein 1 (DDB1), Cullin 4A CUL4A, and DDB1 and Cullin-associated factor 1.^{31,32} The complex then mediates the proteasomal degradation of SAMHD1.^{33,34}

Although HIV-1 lacks Vpx, it can be supplied to cells *in trans* by treatment with Vpx-containing virus-like particles (VLPs), rendering the cells permissive to infection.³⁵ Alternatively, HIV-1 can be engineered to package Vpx by introducing the SIV Vpx-packaging motif into P6 of the Gag polyprotein precursor resulting in a virus that has increased infectivity on MDDCs.³⁶ Incubation of MDDCs with HIV-1 does not induce type-I IFN release or maturation of the cells, but when infection is enhanced by treatment of the cells with Vpx-containing VLPs, the cells sense the newly produced Gag protein following provirus formation.³⁷ The absence of a Vpx gene in HIV-1 was suggested to provide a selective advantage to the virus by limiting the infection of DCs and thereby not triggering an innate immune response. In accordance with this concept, viruses such as HIV-2, SIV_{sm}, and SIV_{agm} which encode a Vpx or Vpr are less pathogenic in their native host.

The ability of Vpx to allow lentivirus infection of myeloid cells is thought to facilitate the ability of the virus to access an important target cell type and to establish a long-lived reservoir. Studies of how lentiviruses infect DCs have been limited to culture-derived MDDCs. Here, we addressed SAMHD1 restriction and the ability of Vpx to counteract the restriction in primary blood pDCs and mDCs. We report that mDCs and pDCs express a high level of SAMHD1. The cells were highly resistant to HIV-1, and Vpx failed to relieve the restriction or induce the SAMHD1 degradation. Blocking interferon signaling partially restored the ability of Vpx to induce the degradation of SAMHD1 and partially relieved the block to infection. The cells responded to incoming virions by activating innate immune response pathways but did not respond to the infection postintegration, suggesting that they lack the sensor that detects newly produced Gag protein in MDDCs.

Materials and Methods

Cells and cell culture

Peripheral blood mononuclear cells (PBMCs) were separated on Ficoll-Hypaque from buffy coat lymphocytes obtained from the New York Blood Center from anonymous donors. The

monocytes were purified from PBMCs by adherence to plastic. Monocytes were differentiated into MDDCs for 4 days in RPMI 1640 containing 5% pooled human serum (PHS) (Innovative Research), 1 mM HEPES, gentamycin, 50 ng/ml GM-CSF (Invitrogen), and 300 U/ml IL-4 (R&D Systems). pDCs were purified from PBMCs by positive selection on anti-BDCA-4-conjugated magnetic beads (Miltenyi Biotec) as previously described³⁸ and were >80% CD123/BDCA-2⁺. The pDCs were cultured in medium supplemented with 10 pg/ml IL-3 (R&D Systems). mDCs were purified from PBMCs by negative selection using an EasySep human mDC enrichment separation system (Stemcell) and were >85% CD11c⁺. CD4⁺ T cells were purified from monocyte-depleted PBMCs by positive selection with anti-CD4 antibody and Flowcomp Dynabeads (Invitrogen) and were >95% CD3⁺ CD4⁺. CD4⁺ T cells were activated by incubation for 3 days with 2.0 µg/ml phytohemagglutinin (PHA) and IL-2 or anti-CD3/CD28-coated Dynabeads (Invitrogen). Resting T cells were <4% CD25⁺, and the activated cells were >80% CD25⁺. Flow cytometry data were analyzed with FlowJo software (Tree Star).

DC maturation

pDCs (2.0×10^5) were stimulated for 16 h with 2.0 µg CpGB (Integrated DNA Technologies). mDCs (2.0×10^5) were stimulated with 5.0 µg/ml poly(I:C) (Amersham). To determine their maturation state, the cells were stained with phycoerythrin (PE)-conjugated anti-CD123 (BD Biosciences) and APC-conjugated anti-CD86, or PE and APC-conjugated isotype control immunoglobulin (Pharmingen), fixed in 1% PFA, and analyzed by flow cytometry. To quantify cytokine and chemokine production, the medium was harvested and replaced 24 and 72 h postinfection. Type-I IFNs were quantified using multisubtype IFN- α and β ELISA kits (PBL Biomedical). To block IFN receptor (IFN-R) signaling, pDCs were incubated for 24 h with a 10 µg/ml mixture of anti-IFN- α , anti-IFN- β , and anti-IFN- α/β receptor chain monoclonal antibodies (PBL Biomedical) or isotype control antimouse IgG₁K.³⁸

Plasmids

pLENTI-GFP³⁹ lentiviral vector and pMDL* HIV-1 Gag/Pol⁴⁰ packaging vector encoding a modified p6 sequence containing the SIV_{mac} Vpx packaging motif have been previously described. pNL4.3-E⁻ CMV-GFP³³ expresses GFP under the control of a CMV promoter. The plasmid was modified to contain the SIV Vpx packaging motif in P6 by excising the *Apa*I to *Eco*-RI fragment of pNL4.3*-luc-E⁻-Vpr⁻³⁶ and replacing the same region of pNL4.3-E⁻ CMV-GFP to generate pNL43*-E⁻-Vpr⁻ CMV-GFP. pcVpx.myc³⁶ encodes a codon-optimized SIV_{mac} Vpx.

Virus preparation and titer

Reporter virus stocks containing or lacking packaged Vpx were produced by calcium phosphate cotransfection of 293T cells with pLenti-CMV-GFP, pMDL*, pcVSVG, pRSV-Rev, and pcVpx.myc or pcDNA6 at a mass ratio of 14:5:3.5:2.5:1. HIV*.CMV.GFP virus stock containing or lacking packaged Vpx was generated by the cotransfection of pNL4.3*-E⁻-Vpr⁻ CMV-GFP, pVSVG, and pcVpx.myc or pcDNA6 at a mass ratio of 4.4:1.8:1. After 6 h, the culture medium was replaced. Virus-containing supernatant was collected 48 h

posttransfection and filtered through a 0.45- μ m pore size membrane. The virus was concentrated 10-fold by ultracentrifugation through 20% sucrose for 90 min at 30,000 RPM in an SW31 Ti rotor (Beckman), resuspended in RPMI, and frozen at -80°C in aliquots. To titer GFP reporter viruses, 293T cells were infected, and after 72 h, the number of GFP⁺ cells was determined by flow cytometry and the titer expressed as the GFP⁺ cell-forming units/ml.

Infection

mDCs, pDCs, and MDDCs (2.0×10^5) were incubated for 16 h with GFP reporter viruses at a multiplicity of infection (MOI) of 2 or 4. Where indicated, the cells were incubated for 24 h with type-I IFN-blocking antibodies before infection. Twenty four hours postinfection, the medium was replaced and used for cytokine profile analysis. The number of infected cells was determined 3 days postinfection by flow cytometry.

Immunoblot analysis

A total of 2.0×10^5 cells were lysed in NP40 lysis buffer (50 mM HEPES, 150 mM KCl, 2 mM EDTA, 0.5% NP-40, and protease inhibitor cocktail) or urea-containing lysis buffer (6 M Urea, 100 mM sodium phosphate, pH 8.0, 20 mM imidazole, pH 8.0). The lysates were normalized for protein concentration by Bradford assay (Bio-Rad). Lysates (5.0 μ g) were separated by SDS-PAGE, transferred to PVDF mem-

branes, and probed with mouse anti-SAMHD1 MAb (Origene), rabbit anti-phosphorylated T592 SAMHD1, rabbit anti-CUL4A antiserum (Abcam), and anti-GAPDH MAb (Ambion) followed by goat anti-mouse or goat anti-rabbit horseradish peroxidase (HRP)-conjugated second antibody (Sigma). The blots were visualized using HRP substrate (Pierce) on an Odyssey imaging system (LI-COR) and the bands were quantified using Licor ImageStudio software. Ratios were calculated as the SAMHD1 signal intensity divided by the GAPDH signal intensity for an identical exposure of the blot.

SAMHD1 degradation

Vpx-induced degradation of SAMHD1 was detected as previously described.³³ Briefly, 2.0×10^5 mDCs, pDCs, or MDDCs were seeded in a 96-well plate. The cells were incubated with Vpx-containing or control virions at the indicated MOI. After 16 h, the cells were lysed in NP40 lysis buffer and SAMHD1 was quantified by immunoblot analysis.

SAMHD1 phosphorylation and CUL4A neddylation

Resting and activated CD4⁺ T cells, mDCs, pDCs, or MDDCs (2.0×10^5) were incubated with 100 U/ml IFN- α_{2a} and IFN- β_{1a} . After 24 h the cells were lysed in urea-containing lysis buffer to prevent deneddylation. The lysates were separated on an SDS-PAGE gel and analyzed by immunoblot to

FIG. 1. Myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) express high levels of SAMHD1 and are resistant to HIV-1 infection. **(A)** SAMHD1 in mDCs, pDCs, and monocyte-derived dendritic cells (MDDCs) from three healthy donors were quantified by immunoblot analysis. The relative abundance of SAMHD1 is expressed as the ratio of SAMHD1 to GAPDH signal intensity under the immunoblot and is represented as a histogram on the right as the average of three donors. **(B)** mDCs, pDCs, and MDDCs were mock infected or infected with HIV-1 GFP reporter virus lacking or containing Vpx. Three days later, the cells were analyzed by flow cytometry. Shown are the FACS plots from a single representative donor (left) and the data in histogram format for the average of three donors (right). Statistical analysis of two **(A)** or three **(B)** independent experiments was performed using two-tailed Student's *t* tests. *Represents a *p*-value < 0.05 and was considered significant.

distinguish the neddylated and nonneddylated forms of CUL4A, as previously described.⁴¹

Results

Primary blood DCs express CD4 and CCR5 yet are relatively resistant to infection by HIV-1. In MDDCs, which are monocytes that have been differentiated *in vitro* with GM-CSF and IL-4, SAMHD1 is at least partially responsible for the restriction. To address the question of whether the block to HIV-1 replication in primary DCs is caused by SAMHD1, we first measured the abundance of protein in healthy donor mDCs, pDCs, and MDDCs. Immunoblot analysis of the purified cell preparations from three donors showed that mDCs and pDCs expressed 6.5- and 3.7-fold more SAMHD1 than MDDCs, a cell type that has relatively high levels of SAMHD1 (Fig. 1A). To determine the extent to which SAMHD1 restricts the infection of DCs, we infected the cells with HIV-1 GFP reporter viruses that contained or lacked packaged SIV_{mac} Vpx. In cells in which infection is restricted by SAMHD1, Vpx is expected to induce SAMHD1 degradation and alleviate the block to infection.

The viruses were produced using a reporter virus into which the SIV_{mac} Vpx packaging motif had been placed in P6 Gag. The virus packages SIV_{mac} Vpx during virus assembly in the producer cell and as a result is much more infectious on cells such as MDDCs that are restricted by SAMHD1.⁴⁰ We infected mDCs, pDCs, and MDDCs with HIV-1 GFP reporter virus that contained or lacked packaged Vpx and, after 3 days, we analyzed the cells by flow cytometry. We found that the MDDCs were resistant to infection with reporter virus lacking Vpx but susceptible to Vpx-containing virus, resulting in 50–60% of the cells becoming infected (Fig. 1B). The mDCs and pDCs, in contrast, were resistant to infection by reporter virus that contained or lacked Vpx, with <5% of the cells on average becoming infected with Vpx-containing virus.

The inability to alleviate the restriction to infection of primary DCs raised the question of whether Vpx had failed to induce SAMHD1 degradation or whether the restriction was mediated by another mechanism. To determine whether Vpx induced the degradation of SAMHD1 in these cells, we infected mDCs, pDCs, and MDDCs with Vpx-containing or control HIV-1 GFP reporter virus at an MOI of 2 and 4, and the following day we quantified SAMHD1 by immunoblot analysis. We found that in MDDCs, Vpx induced the near complete degradation of SAMHD1 at the lower MOI. In contrast, in the mDCs and pDCs, Vpx caused minimal degradation of SAMHD1 even at the higher MOI (Fig. 2). We concluded that there is a block to Vpx function in mDCs and pDCs. This suggests that the cells remain resistant to infection because the SAMHD1 restriction cannot be counteracted by Vpx. It does not rule out the possibility of an additional mechanism of restriction in the DCs.

To further understand the role of SAMHD1 in HIV-1 restriction in the DCs, we analyzed the phosphorylation state of the protein in the cells. T592 has been shown to be a target of CDK1 phosphorylation and plays a key role in regulating the antiviral activity of SAMHD1^{26,27} (although not its catalytic activity²⁶). T592 is antiviral and hypophosphorylated in resting CD4⁺ T lymphocytes. In cycling CD4⁺ T lymphocytes, SAMHD1 T592 is hyperphosphorylated and lacks antiviral activity. In addition, in MDDCs, type-I IFN causes T592

FIG. 2. SAMHD1 in mDCs and pDCs is resistant to Vpx-induced degradation. mDCs, pDCs, and MDDCs were mock infected (m) or infected with HIV-1 GFP reporter virus containing (+) or lacking (-) Vpx at an MOI of 2 or 4. Cell lysates were prepared and SAMHD1 and GAPDH were quantified on an immunoblot. The relative abundance of SAMHD1 is shown below as the ratio of SAMHD1 signal intensity to GAPDH. These data are representative of two independent experiments.

hypophosphorylation, potentiating antiviral activity. To determine the phosphorylation state of SAMHD1 in mDC, pDC, MDDCs, and cycling and noncycling CD4⁺ T cells, we cultured each subpopulation for 24 h in the presence or absence of type-I IFN. To prepare cycling CD4⁺ T cells, we used either PHA and IL-2 or CD3/CD28 costimulation. To evaluate SAMHD1 phosphorylation, we lysed the cells in urea-containing lysis buffer and analyzed the proteins on an immunoblot probed with antiserum specific for the phosphorylated T592 SAMHD1 (Phospho-SAMHD1) (Fig. 3). The results showed that in resting T cells, SAMHD1 was hypophosphorylated and that activation with either PHA and IL-2 or

FIG. 3. SAMHD1 in mDCs and pDCs is neddylated but not phosphorylated on T592. Resting CD4 T cells or CD4 T cells activated with phytohemagglutinin (PHA) and interleukin (IL)-2 or anti-CD3-CD28, mDCs, pDCs, and MDDCs were incubated overnight with or without interferon (IFN)- α_{2a} and IFN- β_{1a} . Cell lysates were prepared and the proteins were analyzed on an immunoblot probed with antibodies to SAMHD1, T592-phosphorylated SAMHD1, CUL4A, and GAPDH. The relative neddylation of CUL4A is shown as the ratio of neddylated CUL4A signal intensity to non-neddylated CUL4A. These data are representative of two independent experiments.

anti-CD3/CD28 induced T592 phosphorylation, consistent with the findings of Cribier *et al.*²⁷ Type-I IFN had no effect on T592 phosphorylation in resting or activated T cells. In mDC and pDC, SAMHD1 was hypophosphorylated. In contrast, in MDDCs the protein was hyperphosphorylated but became hypophosphorylated in response to type-I IFN. The hypophosphorylation of SAMHD1 in the primary DCs is consistent with the protein having strong antiviral activity in these cells.

Cullin-based E3 ubiquitin ligases are regulated by neddylation of the cullin subunit, a process in which the small, ubiquitin-like Nedd8 protein becomes covalently linked to a specific lysine residue of the cullin.⁴² The inability of Vpx to induce the degradation of SAMHD1 in the DCs could be caused by a defect in the function of the CUL4 E3 ubiquitin ligase through which Vpx induces SAMHD1 degradation. To determine whether a lack of CUL4A neddylation could account for the inability of Vpx to induce SAMHD1 degradation in the DCs, we measured the ratio of neddylated to nonneddylated CUL4A in CD4⁺ T lymphocytes, mDC, pDC, and MDDC. In addition, we tested whether type-I IFN affected CUL4A neddylation (Fig. 3). The results showed that in CD4⁺ T cells, the ratio of neddylated to nonneddylated CUL4A was close to 0.8. The ratio was increased to above 1.0 by activation of the cells. The ratio was about 0.6 in mDCs and pDCs and 0.2 in MDDCs. Type-I IFN decreased the neddylation level in mDC and pDC to 0.5 and 0.4, respectively, and did not decrease it in MDDCs. The higher ratio in mDCs and pDCs as compared to MDDCs suggests that neddylation is not a limiting factor for CUL4 function in the cells as the lower ratio in MDDCs is sufficient for Vpx to induce SAMHD1 degradation.

pDCs constitutively express low levels of type-I IFN. Because SAMHD1 is type-I IFN inducible,^{43,44} it is possible that the constitutive type-I IFN production in pDCs contributes to maintaining SAMHD1 expression in these cells by autocrine or paracrine signaling through the type-I IFN-R. To test the dependence of SAMHD1 expression on signaling through the type-I IFN-R, we treated DCs with a type-I IFN-R signaling inhibitory antibody cocktail composed of anti-IFN- α , anti-IFN- β , and anti-type-I IFN-R monoclonal antibodies and then infected the cells with Vpx-containing or control reporter viruses. To verify that the antibody cocktail neutralized free type-I IFN, 24 h posttreatment, we quantified IFN- α in the supernatant (Fig. 4A). The results showed that incubation of virus with pDCs caused the release of type-I IFN, independent of Vpx. The antibody cocktail prevented the virus-induced type-I IFN production.

To determine whether type-I IFN-R signaling was required to maintain the steady-state amount of SAMHD1 in the pDCs, we incubated the pDCs with IFN-R signaling inhibitory cocktail or control immunoglobulin for 24 h and then quantified SAMHD1 by immunoblot analysis. We found that blocking type-I IFN-R signaling did not significantly affect the amount of SAMHD1 expressed in the pDCs (Fig. 4B).

We then tested whether blocking IFN-R signaling would facilitate infection or relieve the block to SAMHD1 degradation. For this, we incubated pDCs with IFN-R signaling inhibitory cocktail and then infected the cells with Vpx-containing or control GFP reporter virus. Three days later, we determined the number of infected cells by flow cytometry. The results showed that HIV-1 infection was blocked in cells infected with control and Vpx-containing virus (Fig. 4C, top).

FIG. 4. HIV-1 virions trigger IFN release by pDCs, which prevents Vpx-induced SAMHD1 degradation. **(A)** pDCs from three healthy donors were incubated for 24 h with type-I IFN-R signaling inhibitory antibody cocktail or isotype control IgG. The cells were then mock infected (m) or infected with HIV-1 GFP reporter virus containing or lacking Vpx. IFN- α was quantified in the supernatant 24 h postinfection by ELISA. **(B)** pDCs were incubated for 24 h with type-I IFN-R signaling inhibitory antibody cocktail or isotype control IgG. Cell lysates were prepared, and SAMHD1 and GAPDH were quantified on an immunoblot. **(C, top)** pDCs from six donors were incubated with IFN-R signaling inhibitory antibody cocktail and then infected as in **(A)**. After 3 days, the percentage of GFP⁺ cells was determined by flow cytometry. Each symbol represents one donor. **(C, bottom)** Sixteen hours postinfection, cell lysates were prepared from one donor and SAMHD1 and GAPDH were quantified on an immunoblot. In **(B)** and **(C)**, the relative abundance of SAMHD1 is shown below the blot defined as the ratio of SAMHD1 signal intensity to GAPDH. Statistical analysis of three **(A)** or six **(C)** independent experiments was performed using two-tailed Student's *t* tests. *Represents a *p*-value < 0.05 and was considered significant.

The antibody cocktail alone failed to relieve the restriction, but when the cells were treated with the antibody cocktail and infected with Vpx-containing virus, the block to infection was partially relieved. To determine whether the antibody cocktail had allowed Vpx to induce the degradation of SAMHD1, we analyzed lysates of the cells on an immunoblot. The results showed that the antibody cocktail allowed for a significant increase in the ability of Vpx to cause the degradation of SAMHD1 (Fig. 4C, bottom). We concluded that constitutive signaling through the type-I IFN-R in pDCs imposes a block to the degradation of SAMHD1.

MDDCs are reported to sense HIV-1 infection through an unidentified cytoplasmic sensor that detects newly produced viral Gag protein following provirus formation, stimulating innate immune response and maturation.³⁷ Whether this sensor is active in primary DCs is not known. To determine whether primary DCs might sense newly produced Gag, we infected the cells at MOI=10, an MOI that was sufficient to allow us to analyze the infected cells. After 24 h and 72 h, we quantified type-I IFN in the supernatant. For this experiment, we infected the cells with virions produced using HIV*-CMV-GFP, an NL4.3-based reporter virus genome that encoded the HIV-1 structural proteins, contained the CMV-GFP cassette in *env*, and had the Vpx packaging motif in P6 Gag. Infection with this virus results in the production of Gag/Pol proteins

from the integrated provirus. Infection of mDCs with Vpx-containing virus resulted in 5% infection (Fig. 5A, left). Application of the virions to the mDCs induced the production of IFN- β 24 h postinfection and the production was independent of Vpx (Fig. 5A, right). At 72 h postinfection, a time at which the provirus is established, IFN- β became undetectable. Infection of pDCs with Vpx-containing virions resulted in 7.5% infected cells (Fig. 5B, left) and similarly induced IFN- α production 24 h postinfection but much less at 72 h. Overall type-I IFN production was about 10-fold higher in pDCs than mDCs, consistent with their known phenotype.³⁸

In addition, we analyzed CD86 and HLA-DR on the infected pDCs by flow cytometry, gating on the GFP⁺ infected population. We found that neither virus induced CD86 (Fig. 5C) or HLA-DR (data not shown). A similar analysis of mDCs could not be done because the cells differentiated spontaneously in culture (data not shown). Taken together, the results suggest that both DC subtypes sense incoming virions by the induction of an innate immune response, but that they do not sense the production of newly produced viral Gag.

Discussion

We report here that primary blood mDCs and pDCs express high levels of SAMHD1 and are resistant to infection by

FIG. 5. Productive infection of mDCs and pDCs by HIV-1 fails to induce innate immune activation. **(A)** mDCs from three healthy donors were mock infected, infected with HIV-1 GFP reporter virus containing (HIV X⁺) or lacking (HIV X⁻) Vpx at an MOI=10, or were treated with poly(I:C) TLR agonist. The number of infected cells was quantified by flow cytometry (left). After 24 h and 72 h, supernatant IFN- β was quantified by ELISA (right). **(B)** pDCs were infected and analyzed as in **(A)** except that CpGB was used as the TLR agonist and IFN- α was quantified. **(C)** pDCs were infected with control or Vpx-containing virus or were treated with CpGB. After 3 days the cells were analyzed by flow cytometry for the maturation marker CD86. Histograms are shown for one representative donor (top). Below, the cells were gated for all GFP⁺ cells, high GFP expressors, and low GFP expressors. The average mean fluorescence intensity (MFI) of three donor cell preparations is shown. Statistical analysis was performed using two-tailed Student's *t* tests. *Represents a *p*-value < 0.05 and was considered significant.

HIV-1. Vpx failed to efficiently counteract the block to infection of mDCs and pDCs and did not induce the degradation of SAMHD1. SAMHD1 was largely hypophosphorylated in the cells, consistent with its high level of antiviral activity. The inability of Vpx to induce SAMHD1 degradation appeared to be caused by the large amount of SAMHD1 and a block to Vpx function.

Our findings suggest that SAMHD1 is a major block to infection of primary DCs. Treating the cells with a cocktail of antibodies against type-I IFN and the type-I IFN-R partially restored the ability of Vpx to induce SAMHD1 degradation and facilitated infection, suggesting that signaling through the type-I IFN-R rendered Vpx unable to induce SAMHD1 degradation. This result is similar to that of Dragin *et al.*⁴⁵ who found that in differentiated THP1 cells, type-I IFN renders Vpx unable to induce SAMHD1 degradation. The mechanism by which type-I IFN blocked the Vpx-induced degradation of SAMHD1 is unclear. We tested whether type-I IFN might prevent the neddylation of CUL4A in the CRL4 E3 ubiquitin ligase, as this would inactivate the ability of the complex to degrade SAMHD1. However, type-I IFN did not decrease the proportion of neddylated CUL4A in mDCs or pDCs to a level lower than that in MDDCs, which is sufficient to allow SAMHD1 degradation.

Our findings suggest that primary DCs are not a major target for HIV-1 or HIV-2 as the cells are restricted for infection by SAMHD1 regardless of whether the virus contains Vpx. Vpx may be more important for allowing the infection of cell types such as macrophages that contain less SAMHD1 and support the Vpx function.^{16,17} *In vivo*, cytokines such as IL-4 and GM-CSF that cause the maturation of myeloid cells may play a role in allowing Vpx to induce SAMHD1 degradation, analogous to what is seen *in vitro* with MDDCs. In addition, Vpx may facilitate the infection of other SAMHD1-restricted cell types such as astrocytes that express SAMHD1 and are thought to be targeted by lentiviruses.^{44,46,47} Although DCs do not become productively infected with HIV-1, they efficiently deliver virions to uninfected T cells through an infectious synapse⁴⁸ and can markedly amplify the infection of T cells. Consistent with previous reports, we found that pDCs in particular respond to virions by secreting a high level of type-I IFN.³⁸ Therefore, the role of DCs in AIDS is more likely to involve the amplification of infection of CD4⁺ T cells and the activation of innate immune pathways in response to incoming virions, rather than in productive infection of DCs themselves.

DCs sense virions by detecting the packaged genomic viral RNA.³⁸ MDDCs have been reported, in addition, to respond to infection at a later time point through an unidentified sensor that detects newly produced Gag.³⁷ Our findings confirm the ability of pDCs and mDCs to rapidly respond to virions by the production of type-I IFN.^{4,5} DCs did not produce type-I IFN 72 h postinfection, a time at which the provirus has formed and new virus proteins are produced, nor did pDCs mature as determined by the absence of increased CD86 or HLA-DR expression. These findings suggest that neither DC subpopulation expresses the cryptic sensor that detects newly produced Gag.³⁷

Dendritic cells play an important role in progression toward AIDS as activators of innate immune pathways in response to virions and in the transfer of virus to CD4⁺ T cells. Their expression of large amounts of SAMHD1 provides them

with a high level of protection from infection by HIV-1 and most likely from other DNA-dependent viruses. Because they are terminally differentiated, nondividing cells, they are likely to be able to withstand the very low levels of dNTPs associated with active SAMHD1. The strong resistance of primary dendritic cells to lentivirus infection will present an obstacle to their use as targets of lentiviral vector-based vaccines.⁴⁰ Vpx in viruses such as SIV_{mac} and HIV-2 serves to increase the tissue tropism of the virus to specific subsets of myeloid cells yet pDCs and mDCs appear to remain resistant to infection. It will be of interest to further understand which cell types Vpx targets for lentivirus infection *in vivo*.

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Author Disclosure Statement

N. Bhardwaj is a coinventor on patents related to DC differentiation and antigen presentation.

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