

# Efficient infection, activation, and impairment of pDCs in the BM and peripheral lymphoid organs during early HIV-1 infection in humanized $\text{rag2}^{-/-}\gamma\text{C}^{-/-}$ mice in vivo

\*Liguo Zhang,<sup>1</sup> \*Qi Jiang,<sup>2,3</sup> Guangming Li,<sup>2</sup> Jerry Jeffrey,<sup>2,4</sup> Grigoriy I. Kovalev,<sup>2</sup> and Lishan Su<sup>1-4</sup>

<sup>1</sup>Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China; <sup>2</sup>Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; <sup>3</sup>Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC; and <sup>4</sup>Curriculum in Genetics and Molecular Biology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

Although plasmacytoid dendritic cells (pDCs) are involved in HIV-1 pathogenesis, the precise mechanism of interaction between pDCs and HIV-1 in vivo is not clear. The conflicting reports in HIV-1-infected patients highlight the importance of studying the interaction between HIV-1 and pDCs in relevant in vivo models. The  $\text{rag2}/\gamma\text{C}$  double knockout (DKO) mouse supports reconstitution of a functional human immune system in central

and peripheral lymphoid organs. We report here that functional pDCs were developed in the BM and peripheral lymphoid organs in humanized DKO (DKO-hu) mice. We show that pDCs from both BM and spleen were activated and productively infected during early HIV infection. The activation level of pDCs correlated with that of  $\text{CD4}^+$  T-cell activation and apoptosis. Although  $\text{CD4}^+$  T cells were preferentially depleted, pDCs were maintained but

functionally impaired in the BM and spleen of HIV-infected DKO-hu mice. We conclude that HIV-1 can efficiently infect, activate, and impair pDCs in the BM and spleen, in correlation with  $\text{CD4}^+$  T-cell depletion. The humanized mouse will serve as a relevant model to investigate the development and function of pDCs and their role during HIV-1 pathogenesis in vivo. (*Blood*. 2011;117(23):6184-6192)

## Introduction

Plasmacytoid dendritic cells (pDCs) are innate immune effector cells that can mature to become APCs and play a key role in bridging innate and adaptive immunity. As innate immune effector cells, pDCs rapidly produce type 1 IFN on exposure to virus infection. After activation/maturation, pDCs will become functional APCs, expressing high levels of MHC and T-cell costimulatory molecules such as CD80 and CD86. Compared with conventional DCs (ie, cDCs or myeloid DCs [mDCs]), activated pDCs also express human inducible costimulator-ligand (ICOS-L) and indoleamine 2,3-dioxygenase, which may contribute to IL-10 expression or T-cell suppression. Therefore, in addition to rapidly producing IFN $\alpha$ , pDCs also function as APCs to up- or down-modulate adaptive immunity, establishing themselves as critical players in coordinating antiviral immunity<sup>1</sup> (and reviewed in Colonna et al<sup>2</sup> and Liu<sup>3</sup>).

Besides high levels of CD4 and other pDC-specific receptors, such as blood dendritic cell antigen (BDCA)2, BDCA4, and immunoglobulin-like transcripts (ILT)7, Toll-like receptor (TLR)7 and TLR9 are preferentially expressed in the endosome of pDC, endowing them as the major sensors of viral RNA and DNA, respectively.<sup>4,5</sup> On exposure to viral RNA or DNA in the endosome, pDCs are rapidly activated to produce type 1 IFN, IL-6, and TNF $\alpha$ . It has been reported that viral RNA (or DNA) binds to TLR7 (or TLR9) to initiate a cascade of signaling events to activate the MyD88-IRAK-TRAF-IRF7 complex. Activated IRF7 migrates into the nucleus to induce expression of IFN genes. In addition, a distinct but overlapping signaling pathway also leads to activation

of IL-6 and TNF $\alpha$  expression, mainly via the NF $\kappa$ B and MAPK pathways. In contrary, cross linking the BDCA2 or ILT7 receptors on pDCs appears to lead to the inhibition of IFN induction via protein tyrosine kinase- and immunoreceptor tyrosine-based activation motif-containing signaling molecules.<sup>6</sup>

The natural ligand for ILT7 has been recently characterized as BM stromal cell antigen 2 (BST2; CD317), an IFN-induced gene.<sup>7</sup> Therefore, the activation of pDCs is modulated by several cell-surface and intracellular receptors to ensure their proper activation and function during host immune responses. HIV-1 Env gp120 interacts with CD4, CXCR4, and CCR5. CD4 binding is proposed to trigger endocytosis to expose the HIV genome to TLR in the endosome.<sup>8,9</sup> Interestingly, the authors of one report suggest that gp120 may bind BDCA2 to affect TLR9-mediated activation of pDCs.<sup>10</sup> In addition, BST2 (aka tetherin) plays an inhibitory role in HIV budding<sup>11</sup> and is degraded or down-regulated by the HIV-encoded protein Vpu to promote HIV virion release from infected target cells. Down-regulation of BST2/tetherin in HIV-1-infected cells may lead to elevated pDC activation because BST2 binds ILT7 to inhibit pDC activation. Therefore, HIV infection can potentially affect pDC activation via multiple mechanisms.

Several lines of evidence have indicated that pDCs may be important in HIV infection and pathogenesis.<sup>2,9</sup> First, pDCs express high levels of CD4, CCR5, and CXCR4, and HIV can productively infect pDCs in vitro. Second, pDCs (but not mDCs) are efficiently activated by HIV in the absence of a productive infection.<sup>9</sup> Third, HIV-positive patients are usually associated with lower levels of

Submitted January 28, 2011; accepted April 9, 2011. Prepublished online as *Blood* First Edition paper, April 19, 2011; DOI 10.1182/blood-2011-01-331173.

\*L.Z. and Q.J. contributed equally to this work.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2011 by The American Society of Hematology

pDC activity. In fact, early study and discovery of human pDCs are determined by the finding that IFN-producing cells are reduced in AIDS patients.<sup>12</sup> The pDCs, therefore, are likely critical modulators of HIV infection and immunopathogenesis.

Pathogenic HIV infections of humans and SIV infections of rhesus macaques are characterized by generalized immune activation and progressive CD4<sup>+</sup> T-cell depletion.<sup>13</sup> In contrast, natural SIV hosts such as sooty mangabeys show a lack of aberrant immune activation, no CD4<sup>+</sup> T-cell depletion, and do not progress to having AIDS, despite high levels of SIV replication. Some early reports support the idea that HIV infection leads to decreased numbers and activity of pDC in the peripheral blood.<sup>14-17</sup> In addition, the nonpathogenic SIV infection in its native host sooty mangabeys is associated with stable levels of pDC, whereas reduced pDC levels are reported in SIV-infected rhesus monkeys during late chronic stages of infection.<sup>18-21</sup>

However, it was recently reported that aberrantly activated pDCs are accumulated in the lymphoid organs during HIV infection. It is proposed that chronic activation of pDCs and IFN production may play a critical role in CD4<sup>+</sup> T-cell depletion and AIDS progression.<sup>22</sup> This hypothesis is supported by recent reports that although both pathogenic and nonpathogenic SIV infection induce pDC activation in acute-phase infection, only nonpathogenic infection is associated with down-regulation of pDC activation.<sup>23-27</sup> The conflicting reports in patients highlight the importance of studying the interaction between HIV and pDCs in relevant models. A robust animal model is urgently needed to study the modulation by, and role of, pDCs in HIV infection.

The Rag2/γC double knockout (DKO) mouse lacks T and B lymphocytes and natural killer cells and serves as an optimal host for the engraftment of human cells/tissues.<sup>28</sup> Remarkably, long-term human T-cell development occurs efficiently in the mouse thymus, and normal human T, B, natural killer, and dendritic cells (both mDC and pDC) are readily detected in the peripheral lymphoid tissues such as spleen, lymph nodes, and peripheral blood. Human T cells developed in the DKO-hu mouse are tolerant to both human and mouse antigens, indicating efficient negative selection by both murine and human APCs. Importantly, de novo human B- and T-cell responses are elicited in the DKO-hu mouse by standard immunization (human TT-specific IgG induction) or infection with the human tumor virus EBV (expansion of EBV-specific CD8 T cells).<sup>28</sup> These EBV-reactive T cells respond to EBV antigens in a human MHC-dependent fashion.<sup>29,30</sup> We and others have shown that HIV infection is efficiently established and persistently detectable in DKO-hu mice with CXCR4, CCR5, or dual-tropic isolates. In addition, human CD4<sup>+</sup> T cells are gradually depleted during HIV infection.<sup>31-33</sup>

We report here that functional pDCs were developed in all lymphoid organs in DKO-hu mice. We show that pDCs from both spleen and BM were productively infected and activated by acute HIV infection. Although CD4<sup>+</sup> T cells were preferentially depleted, pDCs were maintained in lymphoid organs. However, pDCs were functionally impaired in HIV-infected DKO-hu mice. Therefore, HIV infection rapidly activated and impaired pDC functions in lymphoid organs, including the BM. The DKO-hu mouse will serve as a relevant model to investigate the development and function of pDCs and their roles during HIV pathogenesis in vivo.

## Methods

### Construction of DKO-hu mice

DKO-hu mice were constructed as previously reported.<sup>32,33</sup> In brief, human CD34<sup>+</sup> cells were isolated from 17- to 20-week-old fetal liver tissues. The cell suspension released from the liver was filtered through a 70-μm cell strainer (BD Falcon) centrifuged at 150g for 5 minutes to remove hepatocytes. The mononuclear cells were purified through a Ficoll gradient (GE Healthcare Bio-science AB). Cells were labeled with the CD34 MicroBead Kit from Miltenyi Biotec, then CD34<sup>+</sup> cells were positive selected with autoMACS by following the manufacturer's instructions (Miltenyi Biotec). A total of  $1.5 \times 10^5$  CD34<sup>+</sup> HSPC cells were injected into the liver of each DKO mouse at 1-3 days of age, and the mouse has been previously irradiated at 400 rad. Mice who received a transplant were bled through tail vein at 3-4 months after transplantation to check human cell reconstitution by flow cytometry. All animal experiences were reviewed and approved by the University of North Carolina-Chapel Hill Institutional Animal Care and Use Committee.

### HIV infection in DKO-hu mice

We used an HIV molecular clone with a highly pathogenic dual tropic envelope, R3A in NL4-3 backbone,<sup>34</sup> for infection. R3A-HSA was constructed by replacing the *vpr* gene with mouse heat stable antigen (HAS; CD24) as reported.<sup>35</sup> HIV-1 viral stocks were produced in 293T cells and expanded in PHA-activated PBMCs and titered on Hela-CD4-LTR-gal cells (NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID). DKO-hu mice with stable human leukocyte reconstitution were infected with HIV at 4000 infectious units/mouse by intravenous injection. DKO-hu mice that were infected with mock supernatant were included as control groups. HIV replication (genome copy/mL in the plasma) was measured by the Roche Amplicor Monitor v.1.5 qRT-PCR assay (Roche Diagnostics Corporation) or by p24 intracellular staining as previously described.<sup>32,33</sup>

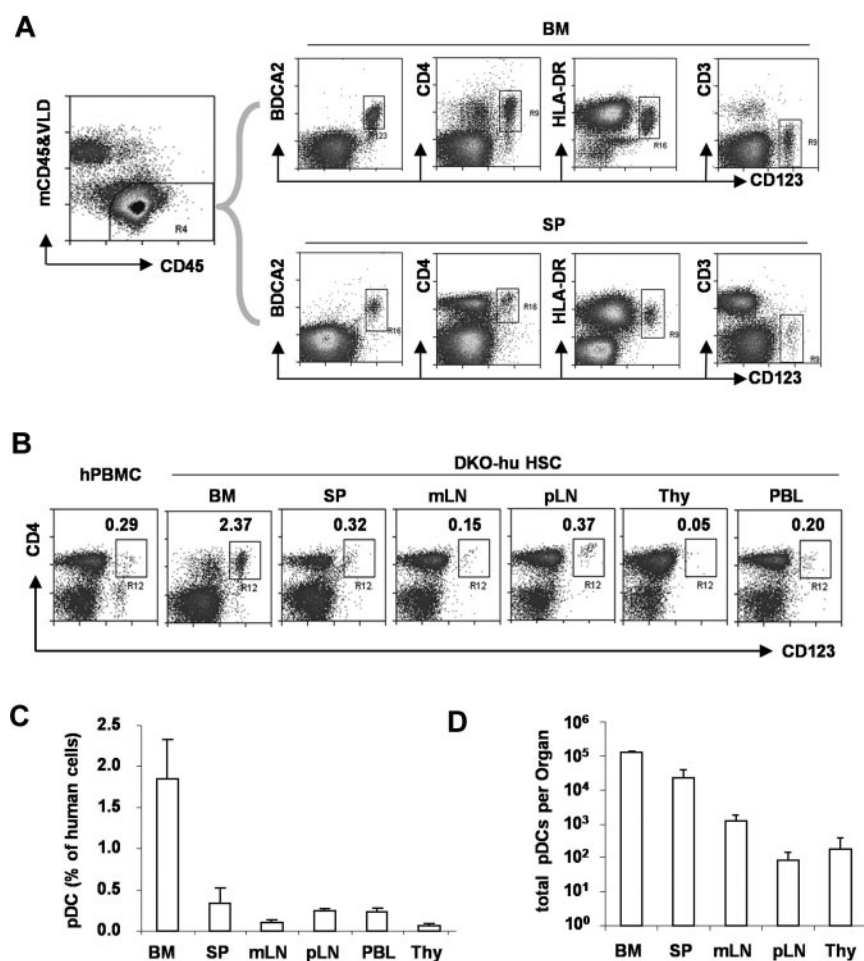
### Flow cytometry

At termination, all lymphoid organs, including thymus, BM, spleen, and lymph nodes, were harvested.<sup>32,33</sup> Total lymphocytes were isolated from mouse lymphoid organs, red blood cells were lysed with ACK buffer, and the remaining cells were stained and fixed with 1% (wt/vol) formaldehyde before analysis. Dead cells were excluded by violet fluorescence dead cell dye (VLD; cat. no. L34955, Invitrogen). Total cell number was quantified by Guava EasyCytos with Guava Express software (Guava). pDC (CD4<sup>+</sup>CD123<sup>+</sup>) numbers were calculated by total cell number from Guava cell counts and percentage of total cells from flow cytometry analysis.

For p24, caspase 3, and IFNα staining, cells were stained with surface antibodies first, then permeabilized with cytofix/cytoperm buffer (BD Bioscience), followed by intracellular staining. Human leukocytes (CD45<sup>+</sup>) were analyzed for CD3, CD4, CD8, CD45RO, CD45RA, HLA-DR, CCR5, and CXCR4 by CyAn FACS machine (Dako). FITC-conjugated anti-human CCR5(2D7), CXCR4(12G5), CD45RA(HI100), and APC-conjugated CD86 were purchased from BD Biosciences; PE/Cy7-anti-human CD3(HIT3a), PE/Cy5-conjugated anti-human CD4(RPA-T4), APC/Cy7-conjugated anti-human CD45, Pacific blue-conjugated anti-mouse CD45(30-F11), PE-conjugated anti-CD80 (2D10), and FITC-conjugated HLA-DR(L243) were purchased from Biolegend; and PE/Texas red-conjugated anti-human CD8 (3B5) antibody and live/dead fixable violet dead cell dye (VLD) were purchased from Invitrogen/Caltag. PE-conjugated anti-human CD303 (BDCA2) was purchased from Miltenyi Biotec. FITC-conjugated anti-HIV p24 (FH190-1-1) was purchased from Beckman Coulter. The cells were analyzed on a Cyan ADP (Dako).

### pDC purification

Total BM cells were stained with biotin-labeled antibody mix to human CD3, CD19, mouse CD45, and mouse Ter119. Lineage-positive cells were



**Figure 1. Development of pDC in lymphoid organs in DKO-hu mice.** (A) Total live human cells (VLD-mCD45<sup>+</sup>hCD45<sup>+</sup>) from BM or spleen (SP) were analyzed for CD123 expression relative to BDCA2, CD4, HLA-DR, and CD3 expression. (B) pDCs in different lymphoid organs from the DKO-hu mice were analyzed. The number in each plot represents the percentage of CD4<sup>+</sup>CD123<sup>+</sup> pDCs of total human CD45<sup>+</sup> cells. Human PBMCs were used as a control. (C) Summarized data show average percentage of pDCs (CD3<sup>+</sup>CD4<sup>+</sup>CD123<sup>+</sup> of total human CD45<sup>+</sup> cells) in different lymphoid organs from DKO-hu mice at 12 weeks after transplantation (n = 7). (D) Total numbers of pDCs in each lymphoid organ are calculated as described in "Flow cytometry." Error bars indicate SD (n = 7).

depleted by streptavidin-labeled magnetic beads with AutoMACS (Miltenyi Biotec). The negative cells were stained with CD4 and CD123. CD4<sup>+</sup>CD123<sup>+</sup> cells were sorted by FACS (> 95% purity).

#### In vitro stimulation of pDCs

Total BM cells ( $2 \times 10^5$  in 100  $\mu$ L of culture medium) or purified pDCs ( $1 \times 10^4$  in 100  $\mu$ L of culture medium) were stimulated with CpG2216 (2  $\mu$ g/mL; InvivoGen), influenza virus A/PR8/34 (2  $\mu$ g/mL; Charles River), or UV-inactivated HSV ( $1 \times 10^7$ /mL, kindly provided by Dr Steven Bachenheimer at University of North Carolina).

#### Human cytokine luminex assay

Cytokines in the mouse plasma or culture supernatant were quantified with Human Cytokine 25-Plex kit (Invitrogen/Biosource). Samples were collected and stored at  $-70^\circ\text{C}$  until assay. Triton X-100 were added (1%, vol/vol) before the assay to inactivate HIV. The assays were performed at the Clinical Proteomics Laboratory at University of North Carolina at Chapel Hill.

#### Statistical analysis

The significance of all comparisons was calculated by use of a Student 2-tailed *t* test assuming unequal variance between mock and HIV-infected groups, and results were considered significant when  $P < .05$ . Correlations between parameters were assessed by the use of the Spearman rank correlation test;  $P < .05$  was considered to be statistically significant.

## Results

### Development of functional human pDCs in central and peripheral lymphoid organs of DKO-hu mice

To define human pDCs from various lymphoid organs, we analyzed human CD45<sup>+</sup> and murine CD45<sup>+</sup> cells in blood or lymphoid tissues from DKO-hu mice. Human pDCs (hCD45<sup>+</sup>CD3<sup>+</sup>CD19<sup>+</sup>CD11c<sup>+</sup>CD4<sup>+</sup>CD123<sup>+</sup>BDCA2<sup>+</sup>) were developed in the BM and other periphery lymphoid organs (Figure 1). Compared with human PBMCs, a similar low frequency of human pDCs was detected in the blood, spleen, or lymph nodes of humanized mice. A relatively greater fraction of human pDCs was detected in the BM, whereas only low numbers of pDCs were detected in the thymus of DKO-hu mice.

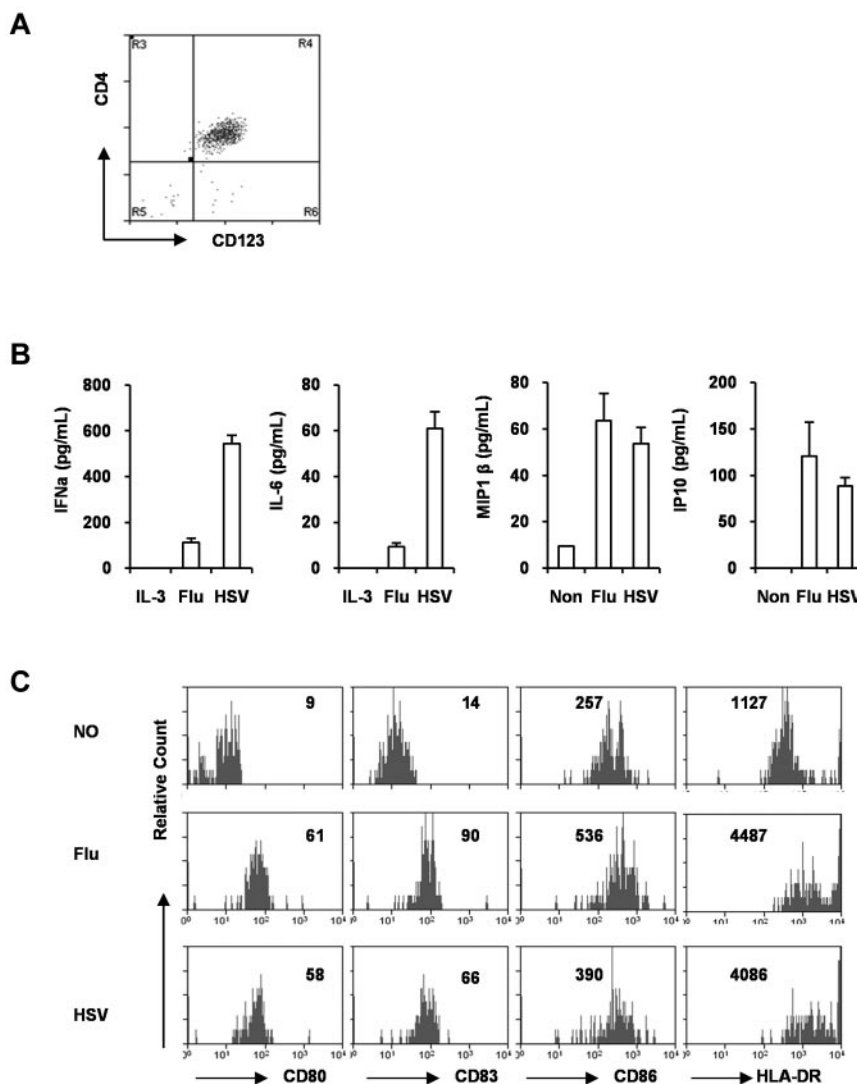
When pDCs from the BM were purified by FACS (Figure 2A), they responded to stimulation with influenza virus (TLR7) and HSV (TLR9) and rapidly produced IFN $\alpha$ , IL-6, and chemokines such as IP10, MIP1 $\beta$ , and IP10 (Figure 2B). In response to TLR7 or TLR9 stimulation, the pDCs also matured to up-regulate CD80, CD83, CD86, HLA-DR, ICOS-L, and other APC receptors (Figure 2C and data not shown). Therefore, pDCs developed in humanized mice were functional in response to TLR7 or TLR9 ligands.

### pDCs in DKO-hu mice express both CCR5 and CXCR and are efficiently infected by HIV in vivo

We showed that human pDCs (Lin<sup>+</sup>CD4<sup>+</sup>CD123<sup>+</sup>) in DKO-hu mice expressed both CCR5 and CXCR4 coreceptors (Figure 3A).

**Figure 2. pDCs from DKO-hu mice are functional.**

(A) pDCs ( $CD45^+CD3^-CD4^+CD123^+$ ) were purified as described in "pDC purification," and the purity was monitored by CD4 and CD123 staining ( $> 95\%$  pure). (B) Purified pDCs (10 000) were cultured in the presence of influenza virus or HSV for 16 hours. Supernatants were collected and  $IFN\alpha$ , IL-6, MIP1 $\beta$ , and IP10 were measured with the Human Cytokine Luminex kit. Error bars are SDs from triplicate samples. (C) Purified pDCs were stimulated with influenza virus or HSV for 48 hours, and then stained with anti-human CD80, CD83, CD86, and HLA-DR monoclonal antibodies. The number in each plot is the mean fluorescence intensity of total pDCs after culture. Flu indicates influenza virus.



We investigated whether pDCs could be directly infected by HIV-1 in the DKO-hu model in various lymphoid organs in vivo. We first measured HIV infection of pDCs by HIV p24 intracellular staining at 1-2 weeks after infection. pDCs were efficiently infected both in spleen and BM. Relative to the infection of human  $CD4^+$  T cells, similar levels of HIV infection of pDCs were detected in the BM of some infected mice (Figure 3B-C). Because the HIV-1 virion-associated p24 may be endocytosed by pDCs, the p24 detected in pDCs did not definitively prove that pDCs were productively infected by HIV-1. We thus further confirmed the productive infection with recombinant HIV-1-expressing mouse heat stable antigen in the *vpr* gene (HIV-R3A-mouse heat stable antigen; Figure 3D). Our data clearly show that HIV-1 efficiently infected pDCs in the BM and spleen during early HIV-1 infection in vivo.

**HIV-1 infection leads to rapid activation of pDCs in vivo**

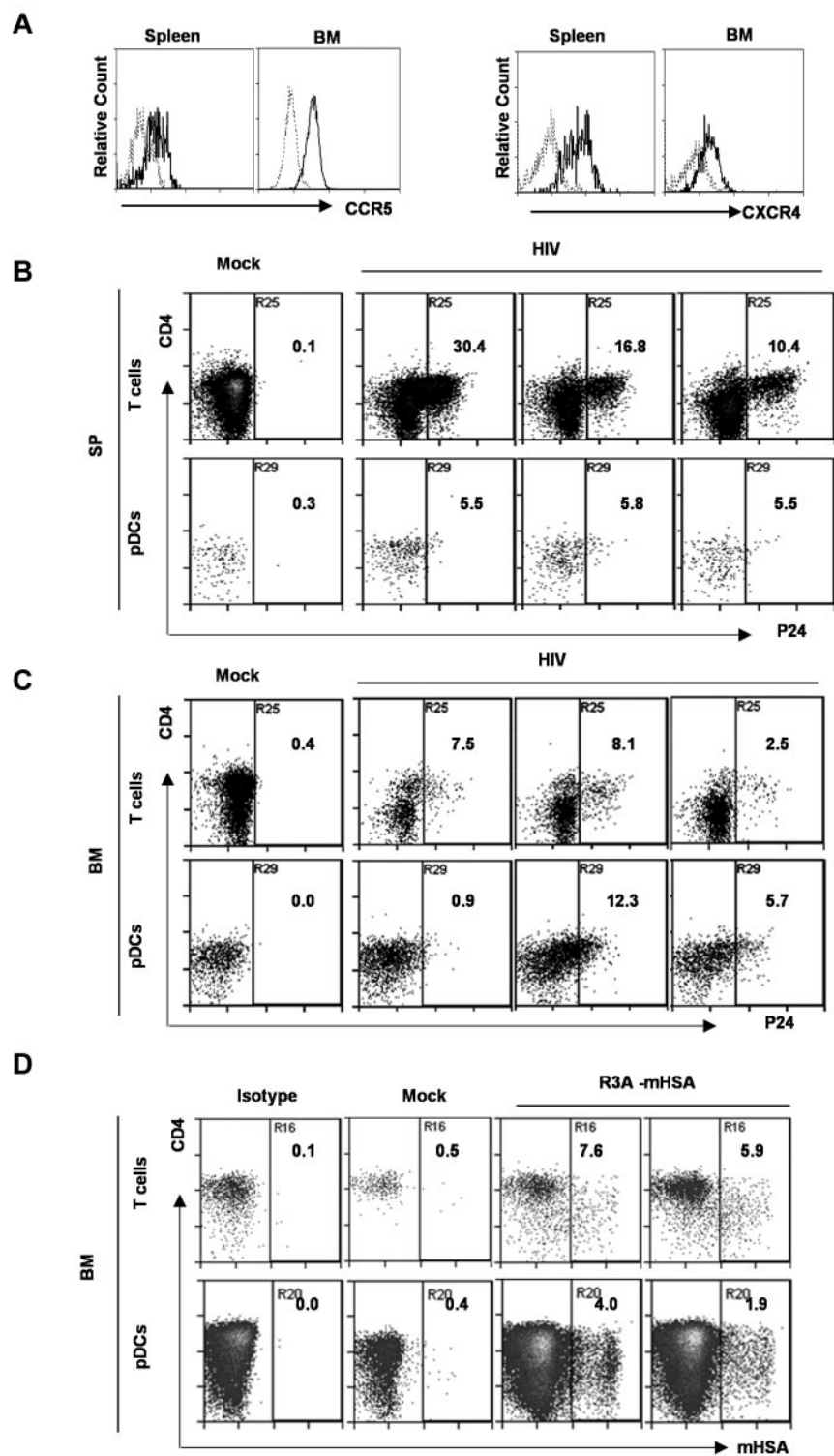
Activation of pDCs is proposed to contribute to the progression of HIV disease.<sup>22</sup> We thus measured the production of  $IFN\alpha$  in the blood from DKO-hu mice infected with mock or HIV. Significant induction of  $IFN\alpha$  was detected in the blood of HIV-infected mice (Figure 4A). In addition, we analyzed the activation markers on pDC from mock and HIV-infected mice. In HIV-infected DKO-hu mice, pDCs were induced to express high levels of HLA-DR,

CD38, CD80, and ICOS-L (Figure 4B-F). Interestingly, the relative activation of pDCs correlated well with  $CD4^+$  T-cell activation (Figure 4G), apoptosis, and depletion (Figure 4H). Thus, human pDCs were rapidly activated in lymphoid organs after HIV-1 infection in DKO-hu mice, and the activated pDCs may contribute to activation and depletion of  $CD4^+$  T cells.

**HIV-1 infection preferentially depletes human  $CD4^+$  T cells but not pDCs in vivo**

To analyze relative depletion of human  $CD4^+$  T cells and pDCs by HIV-1 infection in vivo, we measured the relative frequency and number of human T cells and pDCs in each lymphoid organ. Human  $CD4^+$  T cells were efficiently depleted by HIV infection (in relative frequency and total cell number) in the BM and spleen. In contrast, the frequency of human pDCs was maintained in the BM (Figure 5A,C) and in the spleen (Figure 5D). The total pDC cell number was also maintained in both BM and spleen (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Thus, HIV infection preferentially depleted human  $CD4^+$  T cells but not human  $CD4^+$  pDCs, even though they were also productively infected during HIV infection.





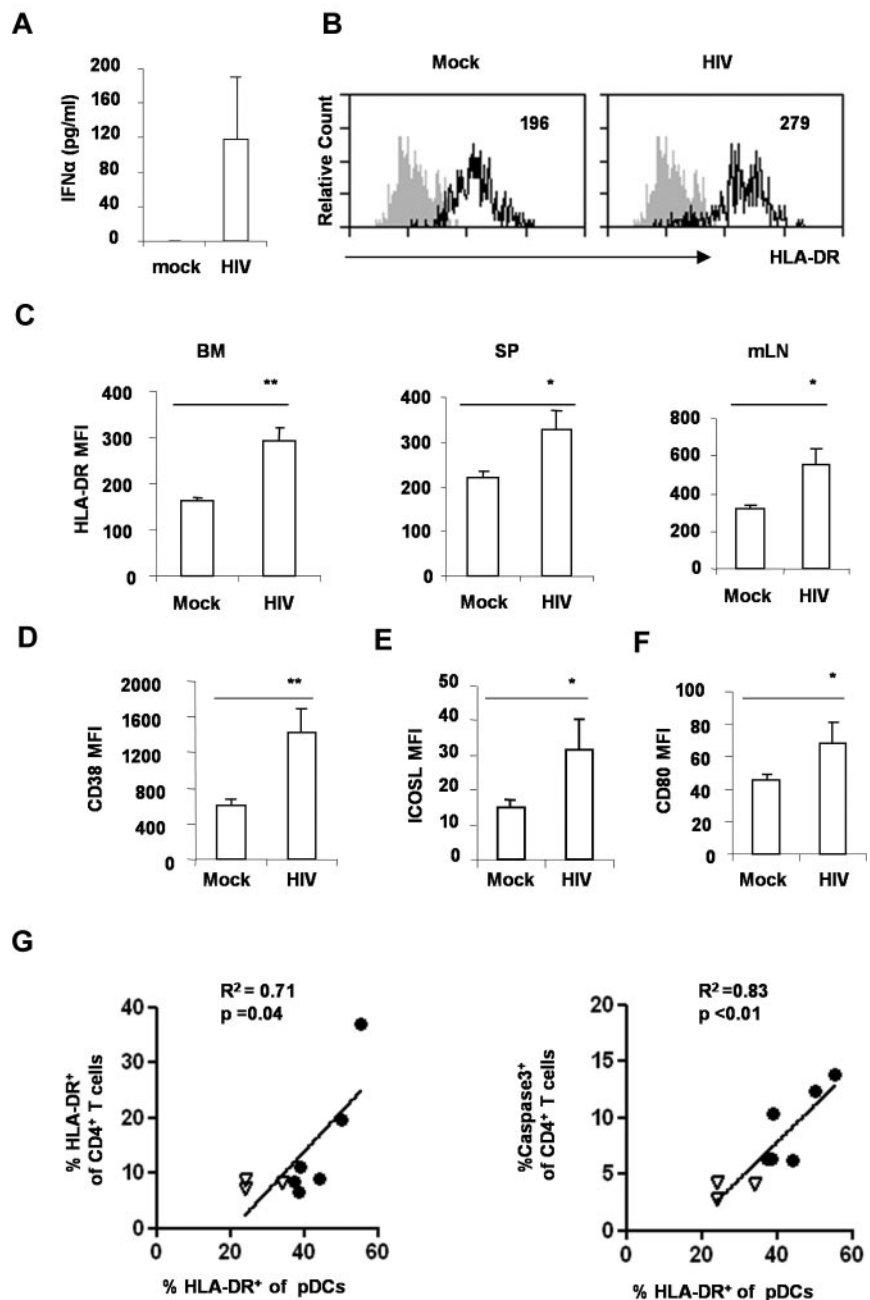
**Figure 3.** pDCs from DKO-hu mice express both HIV coreceptors, and are productively infected by HIV-1 in vivo. (A) Total BM cells or splenocytes were analyzed by FACS. CD3<sup>+</sup>CD4<sup>+</sup>CD123<sup>+</sup> pDCs were further analyzed for CCR5 and CXCR4 expression. Dotted gray lines represent IgG isotype control. (B,C) At 8 days after HIV infection (R3A virus), splenocytes (B) and BM cells (C) were stained with surface markers, followed by HIV p24 intracellular staining. The numbers in the plots represent p24<sup>+</sup> percentage of CD3<sup>+</sup> T cells and CD3<sup>+</sup>BDCA2<sup>+</sup>CD123<sup>+</sup> pDCs. Samples from 1 mock and 3 HIV-infected DKO-hu mice are shown. (D) BM cells from HIV-R3A-HAS-infected DKO-hu mice were analyzed by flow cytometry. The percentage of HSA (mouse CD24) expression on CD3<sup>+</sup>CD8<sup>+</sup> T cells and CD3<sup>+</sup>CD123<sup>+</sup> pDCs from 1 mock and 2 HIV infected DKO-hu mice are shown.

**pDC function is impaired by HIV-1 infection in the BM and spleen in vivo**

Although pDC levels were not reduced in HIV-1-infected DKO-hu mice, the level of IFN $\alpha$  was significantly diminished at 2 wpi in comparison with 1 wpi (Figure 6A-B). We postulated that the pDC function in HIV-1-infected lymphoid organs may be impaired as reported in HIV-1-infected patients. To analyze

the pDC function, we measured the activation of BM pDCs isolated from DKO-hu mice infected with mock or HIV in response to TLR7 or TLR9 ligands. We demonstrated that pDCs from HIV-infected mice were impaired in response to either TLR9 (HSV; Figure 6C-D) or TLR7 (influenza virus; Figure 6E-F) stimulation. We concluded that pDCs in the BM of HIV-1-infected DKO-hu mice were functionally impaired to produce IFN in response to TLR7 and TLR9 stimulation.

**Figure 4. HIV-1 infection induces pDC activation in DKO-hu mice.** (A) Elevated IFN $\alpha$  in HIV-1-infected DKO-hu mice. Plasma from mock or HIV-1-infected mice at 1 week after infection was analyzed for IFN $\alpha$  with the Human Cytokine Luminex kit. IFN $\alpha$  in the mock-infected plasma was lower than the detection limit ( $< 13$  pg/mL). SD is shown as error bar ( $n = 7$  mice). (B) HIV-1 infection activated pDCs in the BM in vivo. BDCA2 $^{+}$ CD123 $^{+}$  pDCs from BM of mock or HIV-infected mice were analyzed for HLA-DR expression by FACS. The number indicates the mean fluorescence intensity (MFI) of total pDCs. Shaded plots are IgG isotype controls. (C) Summarized data show relative expression of HLA-DR on pDCs from BM, spleen (SP), or mesenteric lymph node (mLN) cells. Error bars indicate SDs. pDCs from mock or HIV-infected mice were analyzed for CD38 (D), ICOS-L (E), or CD80 (F) expression. The number is MFI of total pDCs. Shown is summarized data from 3 mock and 4 HIV-infected DKO-hu mice at 2 weeks after HIV infection.  $P$  values between mock and HIV-infected groups were calculated by non-parametric Student  $t$  test. \* $P < .05$ , \*\* $P < .01$ . (G) pDC activation is correlated with CD4 $^{+}$  T-cell activation and apoptosis. BM cells from 3 mock ( $\nabla$ ) and 6 HIV-infected ( $\bullet$ ) mice were analyzed. Cells were stained with surface markers, followed by caspase3 intracellular staining. CD123 $^{+}$ BDCA2 $^{+}$  pDCs and CD3 $^{+}$ CD4 $^{+}$  T cells from BM were analyzed for caspase3 or HLA-DR expression. Correlations were analyzed with the Spearman nonparametric test; squared correlation coefficients ( $R^2$ ), and  $P$  values were shown.

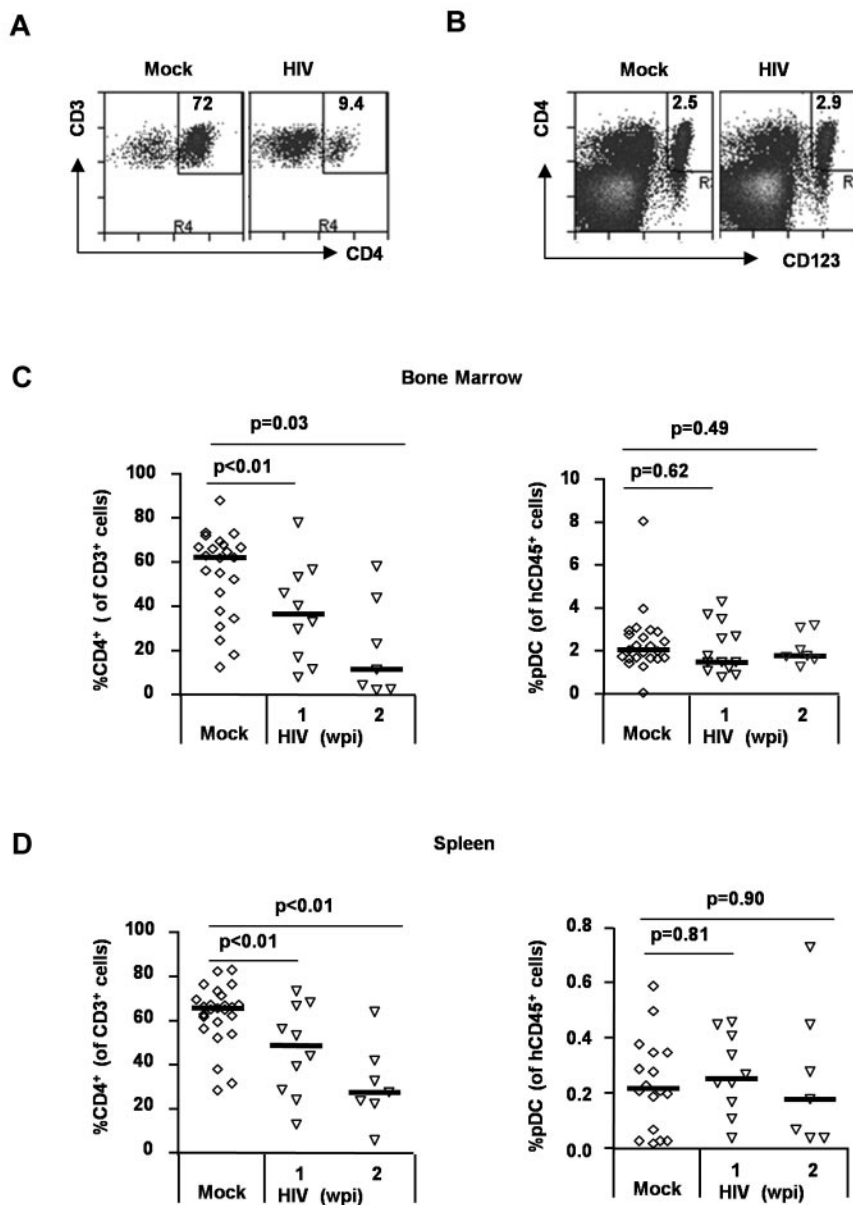


## Discussion

On the basis of findings from HIV-infected humans and SIV-infected monkeys, pDC activation has been implicated in playing a critical role in CD4 $^{+}$  T-cell depletion and AIDS pathogenesis.<sup>19-21</sup> However, the function of human pDC is poorly understood because of its paucity in human peripheral blood and the difficulty of studying pDC in human or monkey lymphoid organs. Here we report that functional pDCs were developed in all lymphoid organs of DKO-hu mice. In addition, HIV-1-infected pDCs efficiently and productively in lymphoid organs in vivo. Interestingly, HIV-1 infection preferentially depleted human CD4 $^{+}$  T cells but only functionally impaired pDCs in the BM and spleen. HIV-1 infection rapidly activated pDCs in both BM and spleen, and relative

activation of pDC correlated with CD4 $^{+}$  T-cell activation and depletion. Our data suggest that the activated but functionally impaired pDCs may contribute to the depletion of CD4 $^{+}$  T cells in HIV-1-infected DKO-hu mice, which will serve as an important model to study development and function of human pDCs in vivo.

Consistent with their expression of CD4 and HIV coreceptors, human pDCs support productive HIV-1 infection in vitro.<sup>15,36-38</sup> HIV-1 antigen or proviral DNA have been detected in pDCs isolated from HIV-1-infected patients.<sup>36,39,40</sup> However, it is not clear whether, and how efficiently, HIV-1 can productively infect pDCs in various lymphoid organs in vivo. Here we report that pDCs were efficiently infected both in the BM and spleen during the early phase of HIV infection. In addition to our direct detection of HIV-1 gag p24 in pDCs with the use of FACS, the HIV-1 virus encoding the murine HSA reporter clearly demonstrated the



**Figure 5. Differential depletion of CD4<sup>+</sup> T cells and pDCs in HIV-infected DKO-hu mice.** (A) Human CD4<sup>+</sup> T cells were depleted by HIV-1 infection. Total BM cells from HIV-infected DKO-hu mice were analyzed by FACS. The numbers in each plot represent the percentage of CD4<sup>+</sup> T cells of total CD3<sup>+</sup> T cells. (B) Human pDCs were not depleted. Total human CD45<sup>+</sup> cells were analyzed for CD4<sup>+</sup>CD123<sup>+</sup> pDC. The number is the percent pDCs of total human CD45<sup>+</sup> cells. (C) Summarized data of human CD4<sup>+</sup> T cells and pDCs in the BM. (D) Summarized data of CD4<sup>+</sup> T cells and pDCs in the spleen. Student *t* test was used to calculate *P* values.

productive infection of pDCs in vivo because HSA expression in target cells depended on productive HIV-1 infection.

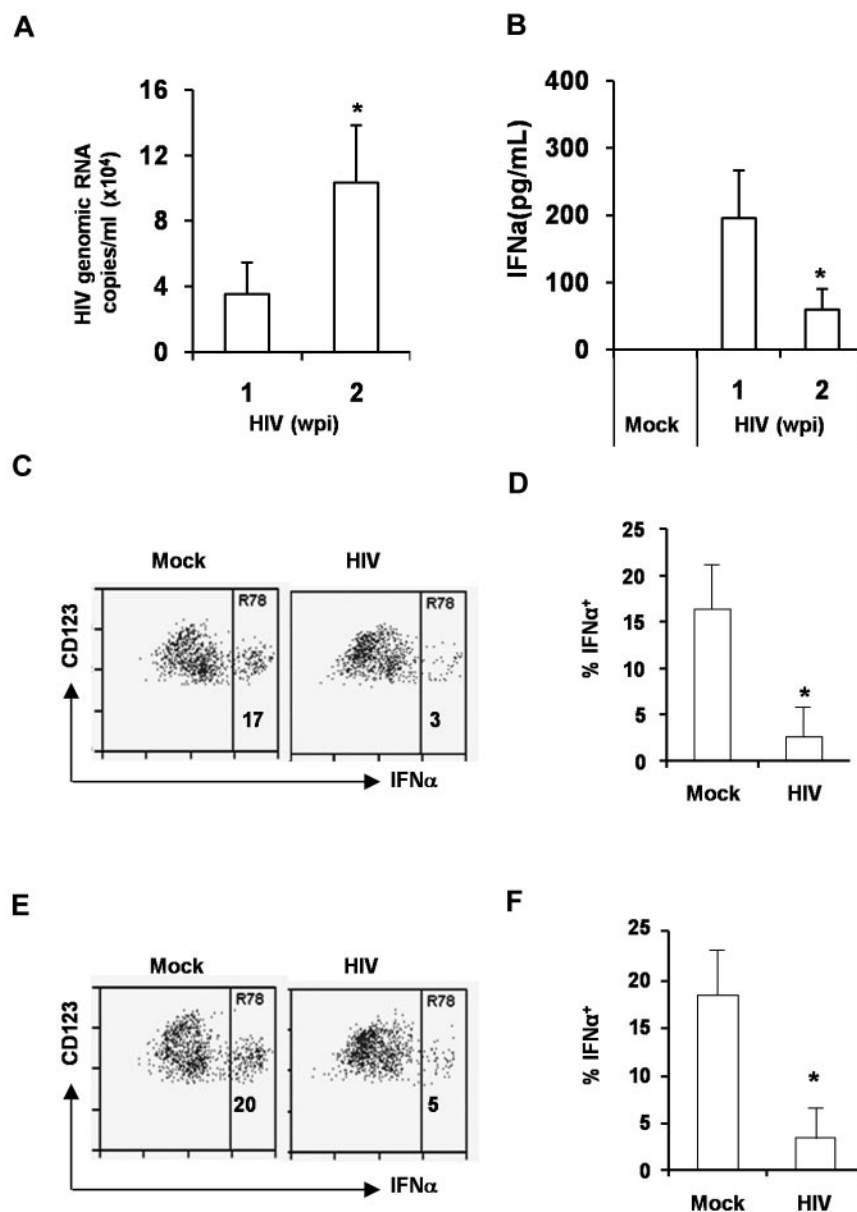
We also showed that, although both efficiently infected, human pDCs in lymphoid organs were not significantly depleted. This finding is consistent with the recent finding that activated pDCs are accumulated in lymph nodes of SIV-infected monkeys<sup>41</sup> and HIV-infected patients.<sup>42-44</sup> However, it has been reported that HIV-1 infection induces apoptosis of pDCs through fusion-dependent mechanisms in vitro.<sup>45</sup> The fate of the HIV-infected pDCs in vivo and their contribution to HIV-1 reservoir will be further investigated in the DKO-hu model.

It has been documented that pDC activity in the blood is impaired or reduced in HIV-infected patients.<sup>17,46,47</sup> We observed that pDCs from the BM of HIV-infected DKO-hu mice also were functionally impaired. However, it has been reported recently that blood pDCs during acute phase of SIV infection in rhesus macaques are functionally normal to TLR7 stimulation.<sup>41</sup> In addition, a recent study also reports that pDCs from the blood of

acute-phase HIV-1 patients are hyperresponsive on TLR7 ligand stimulation, including HIV virions.<sup>48</sup> In an intriguing recent report, pDCs isolated from women show enhanced activation than pDCs isolated from men, which correlates with the preferential AIDS disease progression in HIV-infected women.<sup>49</sup> The discrepancy may be because of the different response of pDCs in the blood and in lymphoid organs, including the BM. Because of the limited human cells in the DKO-hu mouse blood, it is not possible to compare the function of human pDCs in the blood and lymphoid organs of HIV-1-infected DKO-hu mice.

In summary, the role of pDCs in HIV infection and AIDS progression is likely critical but poorly defined. HIV-1 infection-induced aberrant pDC activation may have a deleterious effect on immune system and contribute to disease progression.<sup>22,50</sup> The humanized mouse model with a functional human immune system will serve as a valuable model to study development and function of human pDCs in central and peripheral lymphoid organs in vivo. To “genetically” define the role of pDCs in HIV infection and

**Figure 6. pDCs from HIV infected DKO-hu mice are functionally impaired.** (A) Relative HIV-1 replication level in the blood at 1 and 2 weeks after infection was shown. (B) IFN $\alpha$  in the plasma of mock or HIV-infected DKO-hu mice was measured by the Human Cytokine Luminex kit. Error bars are SD (n = 4). \* $P < .05$ . (C-D) BM cells from mock or HIV-infected DKO-hu mice were stimulated with HSV for 16 hours. The expression of intracellular IFN $\alpha$  in pDCs were measured by FACS (C). (D) Summarized data from 4 mock and 4 HIV-infected DKO-hu mice are shown. (E-F) BM cells were also stimulated with the influenza virus and analyzed for the expression of IFN $\alpha$  in pDCs. (F) Summarized data from 4 mock and 4 HIV infected DKO-hu mice are shown. Error bars represent SD. \* $P < .05$ .



pathogenesis in vivo, a pDC-specific antibody will be useful to deplete pDCs in humanized mice during HIV-1 infection as we have defined the role of Treg cells in HIV-1 infection in the model.<sup>32</sup> Findings regarding HIV-1 interaction with pDCs in vivo and the role of pDCs in HIV disease progression will shed light on the development of a novel therapeutic intervention that targets pDC functions.

## Acknowledgments

We thank Dr Yongjun Liu for discussions and Dedek Brouwer, Selena Barbour, and Anthony Curtis for technical support.

This work was supported in part by grants from the National Natural Science Foundation of China (30872365 to L.Z.), from Ministry of Science and Technology Grants 2006CB910901 and KSCX2-YW-R-150 (to L.Z.); from the Ministry of Health (2009ZX10604 to L.G. and 2008ZX10002-011 to L.S.); and from the National Institutes of Health (R01-AI080432 and R01-

AI077454 to L.S.). We would also like to thank the UNC CFAR, DLAM, and FACS Cores.

## Authorship

Contribution: L.Z., Q.J., and L.S. designed the project, analyzed data, and wrote the paper; and L.Z., Q.J., G.L., J.J., and G.I.K. performed experiments and analyzed data.

Conflict-of-interest disclosure: The authors declare no competing financial interest.

Correspondence: Liguozhang, Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, 15 Da Tun Rd, Chaoyang District, 100101 Beijing, China; e-mail: liguozhang@ibp.ac.cn; and Lishan Su, Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, 15 Da Tun Rd, Chaoyang District, 100101 Beijing, China; e-mail: lsu@med.unc.edu.



## References

- Swiecki M, Gilfillan S, Vermi W, Wang Y, Colonna M. Plasmacytoid dendritic cell ablation impacts early interferon responses and antiviral NK and CD8(+) T cell accrual. *Immunity*. 2010; 33(6):955-966.
- Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. *Nat Immunol*. 2004; 5(12):1219-1226.
- Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol*. 2005;23:275-306.
- Ito T, Wang YH, Liu YJ. Plasmacytoid dendritic cell precursors/type 1 interferon-producing cells sense viral infection by Toll-like receptor (TLR) 7 and TLR9. *Springer Semin Immunopathol*. 2005; 26(3):221-229.
- Lande R, Gregorio J, Facchinetti V, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature*. 2007;449(7162): 564-569.
- Cao W, Rosen DB, Ito T, et al. Plasmacytoid dendritic cell-specific receptor ILT7-Fc epsilonRI gamma inhibits Toll-like receptor-induced interferon production. *J Exp Med*. 2006;203(6):1399-1405.
- Cao W, Bover L, Cho M, et al. Regulation of TLR7/9 responses in plasmacytoid dendritic cells by BST2 and ILT7 receptor interaction. *J Exp Med*. 2009;206(7):1603-1614.
- Beignon AS, McKenna K, Skoberne M, et al. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral RNA interactions. *J Clin Invest*. 2005;115(11):3265-3275.
- Fonteneau JF, Larsson M, Beignon AS, et al. Human immunodeficiency virus type 1 activates plasmacytoid dendritic cells and concomitantly induces the bystander maturation of myeloid dendritic cells. *J Virol*. 2004;78(10):5223-5232.
- Martinelli E, Cicala C, Van Ryk D, et al. HIV-1 gp120 inhibits TLR9-mediated activation and IFN- $\alpha$  secretion in plasmacytoid dendritic cells. *Proc Natl Acad Sci U S A*. 2007;104(9):3396-3401.
- Perez-Caballero D, Zang T, Ebrahimi A, et al. Tetherin inhibits HIV-1 release by directly tethering virions to cells. *Cell*. 2009;139(3):499-511.
- Siegal FP, Kadowaki N, Shodell M, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science*. 1999;284(5421): 1835-1837.
- Giorgi JV, Nishanian PG, Schmid I, Hultin LE, Cheng HL, Detels R. Selective alterations in immunoregulatory lymphocyte subsets in early HIV (human T-lymphotropic virus type III/lymphadenopathy-associated virus) infection. *J Clin Immunol*. 1987;7(2):140-150.
- Siegal FP, Fitzgerald-Bocarsly P, Holland BK, Shodell M. Interferon-alpha generation and immune reconstitution during antiretroviral therapy for human immunodeficiency virus infection. *Aids*. 2001;15(13):1603-1612.
- Donaghy H, Pozniak A, Gazzard B, et al. Loss of blood CD11c(+) myeloid and CD11c(-) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load. *Blood*. 2001;98(8):2574-2576.
- Pacanowski J, Kahi S, Baillet M, et al. Reduced blood CD123+ (lymphoid) and CD11c+ (myeloid) dendritic cell numbers in primary HIV-1 infection. *Blood*. 2001;98(10):3016-3021.
- Feldman S, Stein D, Amrute S, et al. Decreased interferon-alpha production in HIV-infected patients correlates with numerical and functional deficiencies in circulating type 2 dendritic cell precursors. *Clin Immunol*. 2001;101(2):201-210.
- Brown KN, Trichel A, Barratt-Boyes SM. Parallel loss of myeloid and plasmacytoid dendritic cells from blood and lymphoid tissue in simian AIDS. *J Immunol*. 2007;178(11):6958-6967.
- Diop OM, Ploquin MJ, Mortara L, et al. Plasmacytoid dendritic cell dynamics and alpha interferon production during Simian immunodeficiency virus infection with a nonpathogenic outcome. *J Virol*. 2008;82(11):5145-5152.
- Reeves RK, Fultz PN. Disparate effects of acute and chronic infection with SIVmac239 or SHIV-89.6P on macaque plasmacytoid dendritic cells. *Virology*. 2007;365(2):356-368.
- Malleret B, Karlsson I, Maneglier B, et al. Effect of SIVmac infection on plasmacytoid and CD1c+ myeloid dendritic cells in cynomolgus macaques. *Immunology*. 2008;124(2):223-233.
- Herbeuval JP, Shearer GM. HIV-1 immunopathogenesis: how good interferon turns bad. *Clin Immunol*. 2007;123(2):121-128.
- Harris LD, Tabb B, Sodora DL, et al. Downregulation of robust acute type I interferon responses distinguishes nonpathogenic simian immunodeficiency virus (SIV) infection of natural hosts from pathogenic SIV infection of rhesus macaques. *J Virol*. 2010;84(15):7886-7891.
- Campillo-Gimenez L, Laforge M, Fay M, et al. Nonpathogenesis of simian immunodeficiency virus infection is associated with reduced inflammation and recruitment of plasmacytoid dendritic cells to lymph nodes, not to lack of an interferon type I response, during the acute phase. *J Virol*. 2010;84(4):1838-1846.
- Manches O, Bhardwaj N. Resolution of immune activation defines nonpathogenic SIV infection. *J Clin Invest*. 2009;119(12):3512-3515.
- Jacquelin B, Mayau V, Targat B, et al. Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I IFN response. *J Clin Invest*. 2009;119(12):3544-3555.
- Lederer S, Favre D, Walters KA, et al. Transcriptional profiling in pathogenic and non-pathogenic SIV infections reveals significant distinctions in kinetics and tissue compartmentalization. *PLoS Pathog*. 2009;5(2):e1000296.
- Traggiai E, Chicha L, Mazzucchelli L, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science*. 2004; 304(5667):104-107.
- Shultz LD, Saito Y, Najima Y, et al. Generation of functional human T-cell subsets with HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL2r gamma(null) humanized mice. *Proc Natl Acad Sci U S A*. 2010; 107(29):13022-13027.
- Strowig T, Gurer C, Ploss A, et al. Priming of protective T cell responses against virus-induced tumors in mice with human immune system components. *J Exp Med*. 2009;206(6):1423-1434.
- Baenziger S, Tussiwand R, Schlaepfer E, et al. Disseminated and sustained HIV infection in CD34+ cord blood cell-transplanted Rag2-/- gamma c-/- mice. *Proc Natl Acad Sci U S A*. 2006; 103(43):15951-15956.
- Jiang Q, Zhang L, Wang R, et al. FoxP3+CD4+ Treg cells play an important role in acute HIV-1 infection in humanized rag2-/- (gamma)C-/- mice in vivo. *Blood*. 2008;112(7):2858-2868.
- Zhang L, Kovalev GI, Su L. HIV-1 infection and pathogenesis in a novel humanized mouse model. *Blood*. 2007;109(7):2978-2981.
- Meissner EG, Coffield VM, Su L. Thymic pathogenicity of an HIV-1 envelope is associated with increased CXCR4 binding efficiency and V5-gp41-dependent activity, but not V1/V2-associated CD4 binding efficiency and viral entry. *Virology*. 2005;336(2):184-197.
- Jamieson BD, Zack JA. In vivo pathogenesis of a human immunodeficiency virus type 1 reporter virus. *J Virol*. 1998;72(8):6520-6526.
- Fong L, Mengozzi M, Abbey NW, Herndier BG, Engleman EG. Productive infection of plasmacytoid dendritic cells with human immunodeficiency virus type 1 is triggered by CD40 ligation. *J Virol*. 2002;76(21):11033-11041.
- Yonezawa A, Morita R, Takaori-Kondo A, et al. Natural alpha interferon-producing cells respond to human immunodeficiency virus type 1 with alpha interferon production and maturation into dendritic cells. *J Virol*. 2003;77(6):3777-3784.
- Smed-Sørensen A, Lore K, Vasudevan J, et al. Differential susceptibility to human immunodeficiency virus type 1 infection of myeloid and plasmacytoid dendritic cells. *J Virol*. 2005;79(14): 8861-8869.
- Donaghy H, Gazzard B, Gotch F, Patterson S. Dysfunction and infection of freshly isolated blood myeloid and plasmacytoid dendritic cells in patients infected with HIV-1. *Blood*. 2003;101(11): 4505-4511.
- Schmidt B, Scott I, Whitmore RG, et al. Low-level HIV infection of plasmacytoid dendritic cells: onset of cytopathic effects and cell death after PDC maturation. *Virology*. 2004;329(2):280-288.
- Brown KN, Wijewardana V, Liu X, Barratt-Boyes SM. Rapid influx and death of plasmacytoid dendritic cells in lymph nodes mediate depletion in acute simian immunodeficiency virus infection. *PLoS Pathog*. 2009;5(5):e1000413.
- Nascimbene M, Perie L, Chorro L, et al. Plasmacytoid dendritic cells accumulate in spleens from chronically HIV-infected patients but barely participate in interferon-alpha expression. *Blood*. 2009;113(24):6112-6119.
- Dillon SM, Robertson KB, Pan SC, et al. Plasmacytoid and myeloid dendritic cells with a partial activation phenotype accumulate in lymphoid tissue during asymptomatic chronic HIV-1 infection. *J Acquir Immune Defic Syndr*. 2008;48(1):1-12.
- Lehmann C, Lafferty M, Garzino-Demo A, et al. Plasmacytoid dendritic cells accumulate and secrete interferon alpha in lymph nodes of HIV-1 patients. *PLoS One*. 2010;5(6):e11110.
- Meyers JH, Justement JS, Hallahan CW, et al. Impact of HIV on cell survival and antiviral activity of plasmacytoid dendritic cells. *PLoS ONE*. 2007; 2(5):e458.
- Howell DM, Feldman SB, Kloser P, Fitzgerald-Bocarsly P. Decreased frequency of functional natural interferon-producing cells in peripheral blood of patients with the acquired immune deficiency syndrome. *Clin Immunol Immunopathol*. 1994;71(2):223-230.
- Tilton JC, Manion MM, Luskin MR, et al. Human immunodeficiency virus viremia induces plasmacytoid dendritic cell activation in vivo and diminished alpha interferon production in vitro. *J Virol*. 2008;82(8):3997-4006.
- Sabado RL, O'Brien M, Subedi A, et al. Evidence of dysregulation of dendritic cells in primary HIV infection. *Blood*. 2010;116(19):3839-3852.
- Meier A, Chang JJ, Chan ES, et al. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. *Nat Med*. 2009;15(8):955-959.
- Stary G, Klein I, Kohlhofer S, et al. Plasmacytoid dendritic cells express TRAIL and induce CD4+ T-cell apoptosis in HIV-1 viremic patients. *Blood*. 2009;114(18):3854-3863.