Efficient Infection, Activation and Impairment of pDC in the Bone Marrow and Peripheral Lymphoid Organs during early HIV-1 Infection in Humanized rag2\(^{-/-}\)γC\(^{-/-}\) Mice in vivo

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Abstract

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 rag2/γC 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 bone marrow and peripheral lymphoid organs in humanized DKO (DKO-hu) mice. We show that pDCs from both bone marrow and spleen were activated and productively infected during early HIV infection. The activation level of pDCs correlated with that of CD4+ T cell activation and apoptosis. While CD4+ T cells were preferentially depleted, pDCs were maintained but functionally impaired in the bone marrow and spleen of HIV-infected DKO-hu mice. We conclude that HIV-1 can efficiently infect, activate and impair pDCs in the bone marrow and spleen, correlated with 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.
Introduction

Plasmacytoid dendritic cells (pDC) are innate immune effector cells that can mature to become antigen-presenting cells (APC), and play a key role in bridging innate and adaptive immunity. As innate immune effector cells, pDCs rapidly produce type 1 IFN upon exposure to virus infection. After activation/maturation, pDCs will become functional APC expressing high levels of MHC and T cell costimulatory molecules such as CD80 and CD86. Compared with conventional DC (cDC or mDC), activated pDCs also express human inducible costimulator-ligand (ICOS-L) and indoleamine 2,3-dioxygenase (IDO) that may contribute to IL-10 expression or T cell suppression. Therefore, in addition to rapidly producing IFNα, pDCs are also function as APC to up- or down-modulate adaptive immunity, establishing themselves as critical players in coordinating anti-viral immunity1 (and reviewed in 2-3).

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 4-5. Upon exposure to viral RNA or DNA in the endosome, pDCs are rapidly activated to produce type 1 interferon (IFN), IL-6 and TNFα. 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 TNFα expression, mainly via the NFκB and MAPK pathways. In contrary, cross linking the BDCA2 or ILT7 receptors on pDCs appear to lead to inhibition of IFN induction via PTK and ITAM-containing signaling molecules 6. The natural ligand for ILT7 has been recently characterized as bone marrow stromal cell antigen 2 (BST2; CD317), an IFN-induced gene 7. Therefore, activation of pDCs are modulated by a number of 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 HIV genome to TLR in the endosome 8-9. Interestingly, one report suggests that gp120 may bind BDCA2 to affect TLR9 mediated activation of pDCs 10. In addition, BST2 (aka Tetherin) plays an inhibitory role in HIV budding 11, and is degraded or down-regulated by 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 since 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. First, pDCs express high levels of CD4, CCR5 and CXC4 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. Third, HIV+ patients are usually associated with lower levels of pDC activity. In fact, early study and discovery of human pDCs are based on the finding that IFN-producing cells are reduced in AIDS patients. The pDCs, therefore, are likely critical modulators of HIV infection and immuno-pathogenesis.

Pathogenic HIV infections of humans and simian immunodeficiency virus (SIV) infections of rhesus macaques are characterized by generalized immune activation and progressive CD4+ T cell depletion. In contrast, natural SIV hosts such as sooty mangabeys show a lack of aberrant immune activation, no CD4+ T cell depletion and do not progress to AIDS, despite high levels of SIV replication. Some early reports support the idea that HIV infection leads to declined pDC number and activity in peripheral blood. In addition, the non-pathogenic SIVsm 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 (RM) during late chronic stages of infection. However, it is 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+ T cell depletion and AIDS progression. This is supported by recent reports that even though both pathogenic and nonpathogenic SIV infection induce pDC activation in acute phase infection, only nonpathogenic infection is associated with down-regulation of pDC activation. 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, B lymphocytes and NK cells, and serves as optimal hosts for engraftment of human cells/tissues. Remarkably, long term human T cell development occurs efficiently in the mouse thymus, and normal human T, B, NK and dendritic cells (both mDC and pDC) are readily detected in peripheral lymphoid tissues such as spleen, lymph nodes (LN) and peripheral blood (PB). Human T cells developed in the DKO-hu mouse are tolerated to both human and mouse antigens, indicating efficient negative selection by both murine and human APC. 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)\textsuperscript{28}. These EBV-reactive T cells respond to EBV antigens in a human MHC-dependent fashion\textsuperscript{29-30}. Others and we 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\textsuperscript{+} T cells are gradually depleted during HIV infection\textsuperscript{31-33}.

We report here that functional pDCs were developed in all lymphoid organs in DKO-hu mice. We show that pDCs from both spleen and bone marrow were productively infected and activated by acute HIV infection. While CD4\textsuperscript{+} 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 bone marrow. 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.
Material and Methods

Construction of DKO-hu mice
DKO-hu mice were constructed as previously reported\(^\text{32-33}\). Briefly, human CD34\(^+\) cells were isolated from 17-20 weeks old fetal liver tissues. The cell suspension released from the liver was filtered through a 70-um cell strainer (BD Falcon) centrifuged at 150xg for 5 min to get rid of hepatocytes. The mononuclear cells were purified through Ficoll gradient (GE Healthcare Bioscience AB). Cells were labeled with CD34 MicroBead Kit from Miltenyi Biotec, then CD34\(^+\) cells were positive selected with autoMACS followed the vendor’s instruction (Miltenyi Biotec, Germany). 1-5x10\(^5\) CD34\(^+\) HSPC cells were injected into the liver of each DKO mouse at 1 to 3 days old, which has been previously irradiated at 400 rad. Transplanted mice were bled through tail vein at 3-4 months after transplant 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\(^\text{34}\) for infection, R3A-HSA was constructed by replacing vpr gene with mouse HSA (CD24) as reported\(^\text{35}\). HIV-1 viral stocks were produced in 293T cells and expanded in PHA activated PBMC, 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 intravenously injection. DKO-hu mice 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, Indianapolis, IN), or by p24 intracellular staining as previously described\(^\text{32-33}\).

Flow cytometry
At termination, all lymphoid organs including thymus, bone marrow, spleen, lymph nodes were harvested\(^\text{32-33}\). 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% (W/V) formaldehyde before analysis. Dead cells were excluded by violet fluorescence dead cell dye (VLD) (L34955, Invitrogen). Total cell number was quantified by Guava EasyCytes with Guava
Express software (Guava, Hayward, CA). pDC (CD4+CD123+) 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 stain with surface antibodies first, then permeabilized with cytofix/cytperm buffer (BD Bioscience), followed by intracellular staining. Human leukocytes (CD45+) were analyzed for CD3, CD4, CD8, CD45RO, CD45RA, HLA-DR, CCR5, and CXCR4 by CyAn FACS machine (Dako North America, Inc, Carpinteria, CA). FITC-conjugated anti-human CCR5(2D7), CXCR4(12G5), CD45RA(HI100) and APC conjugated CD86 were purchased from BD Biosciences (San Diego, CA); 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), FITC conjugated HLA-DR(L243), were purchased from Biolegend (San Diego, CA); PE/Texas red conjugated anti human CD8 (3B5) antibody and Live/dead fixable violet dead cell dye (VLD) were purchased from Invitrogen/Caltag (Invitrogen, Carlsbad, CA). PE conjugated anti human CD303 (BDCA2) was purchased from Miltenyi (Miltenyi Biotech, Germany). FITC-conjugated anti-HIV p24 (FH190-1-1) was purchased from Beckman Coulter (Fullerton, CA). The cells were analyzed on a Cyan ADP (DAKO, Denmark).

**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 depleted by streptavidin labeled magnetic beads with AutoMACS (Miltenyi Biotech, Germany). The negative cells were stained with CD4 and CD123. CD4+CD123+ cells were sorted by FACS (>95% purity).

**In vitro stimulation of pDCs**

Total bone marrow cells (2×10^5 in 100 μl culture medium) or purified pDCs (1×10^4 in 100 μl culture medium) were stimulated with CpG2216 (2 μg/ml, InvivoGen, San Diego, CA), influenza virus A/PR8/34 (2 μg/ml, Charles River), or UV inactivated HSV (1×10^7/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, Carlsbad, CA). Samples were collected and stored at –70°C until assay. Triton X-100 were added (1%, Vol/Vol) before 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 using a Student’s two-tailed $t$ test assuming unequal variance between mock and HIV-infected groups, and results considered significant when $p < 0.05$. Correlations between parameters were assessed using Spearman’s rank correlation test, $P$ values $< 0.05$ were 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⁺ and murine CD45⁻ cells in blood or lymphoid tissues from DKO-hu mice. Human pDCs (hCD45⁺ CD3⁻ CD19⁻ CD11c⁻ CD4⁺ CD123⁺ BDCA2⁺) were developed in the bone marrow and other periphery lymphoid organs (Fig. 1). Compared with human PBMC, similar low frequency of human pDC was detected in the blood, spleen or lymph nodes of humanized mice. A relatively higher fraction of human pDC was detected in the bone marrow, whereas only low numbers of pDCs were detected in the thymus, of DKO-hu mice. When pDCs from the bone marrow were purified by FACS (Fig. 2A), they responded to stimulation with influenza virus (TLR7) and HSV (TLR9), and rapidly produced IFNα, IL-6 and chemokines such as IP10, MIP1β and IP10 (Fig. 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 (Fig. 2C and data not shown). Therefore, pDC 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⁻CD4⁺CD123⁺) in DKO-hu mice expressed both CCR5 and CXCR4 coreceptors (Fig. 3A). We investigated if pDCs can 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 post infection. pDCs were efficiently infected both in spleen and bone marrow. Relative to infection of human CD4⁺ T cells, similar levels of HIV infection of pDCs were detected in the bone marrow of some infected mice (Fig. 3B&C). Since the HIV-1 virion associated p24 may be endocytosed by pDC, 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 (mHSA) in the vpr gene (HIV-R3A-mHSA, Fig. 3D). Our data clearly show that HIV-1 efficiently infected pDCs in the bone marrow and spleen during early HIV-1 infection in vivo.

**HIV-1 infection leads to rapid activation of pDC in vivo.** Activation of pDCs is proposed to contribute to HIV disease progression. We thus measured production of IFNα in the blood from DKO-hu mice infected with mock or HIV. Significant induction of IFNα was detected in the blood of HIV-infected mice (Fig. 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 (Fig. 4B-F). Interestingly, the relative activation of pDCs correlated well with CD4⁺ T cell activation (Fig. 4G), apoptosis and depletion (Fig. 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 bone marrow and spleen. In contrast, the frequency of human pDCs were maintained in the bone marrow (Fig. 5A & 5C) and in the spleen (Fig. 5D). The total pDC cell number was also maintained in both bone marrow and spleen (Fig. S1). Thus, HIV infection preferentially depleted human CD4⁺ T cells but not human CD4⁺ pDCs even though they were also productively infected during HIV infection.

**pDC function is impaired by HIV-1 infection in the bone marrow and spleen in vivo.** Although pDC levels were not reduced in HIV-1 infected DKO-hu mice, the level of IFNα was significantly diminished at 2 wpi in comparison to 1 wpi (Fig. 6 A&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 bone marrow pDC 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, Fig. 6 C&D) or TLR7 (influenza virus, Fig. 6 E& F) stimulation. We conclude that pDCs in the bone marrow of HIV-1 infected DKO-hu mice were functionally impaired to produce IFN in response to TLR7 and TLR9 stimulation.
Discussion

Based on 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 pathogenesis19-21. However, the function of human pDC is poorly understood due to 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 bone marrow and spleen. HIV-1 infection rapidly activated pDCs in both bone marrow 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 co-receptors, human pDCs support productive HIV-1 infection in vitro15,36-38. HIV-1 antigen or proviral DNA have been detected in pDCs isolated from HIV-1 infected patients36,39-40. However, it is not clear if, 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 bone marrow and spleen during early phase of HIV infection. In addition to direct detection of HIV-1 gag p24 in pDCs by FACS, the HIV-1 virus encoding the murine HSA reporter clearly demonstrated the 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 is consistent with the recent finding that activated pDCs are accumulated in lymph nodes of SIV-infected monkeys41 and HIV-infected patients42,43,44. However, it has been reported that HIV-1 infection induces apoptosis of pDCs through fusion-dependent mechanisms in vitro45. 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 patients17,46,47. We observed that pDCs from the bone marrow of HIV infected DKO-hu mice were also 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 \(^{41}\). In addition, a recent study also reports that pDCs from blood of acute phase HIV-1 patients are hyper-responsive upon TLR7 ligand stimulation, including HIV virions \(^{48}\). In an intriguing recent report, pDCs isolated from women show enhanced activation than pDCs isolated from men, correlated with the preferential AIDS disease progression in HIV-infected women \(^{49}\). The discrepancy may be due to the different response of pDCs in the blood and in lymphoid organs including the bone marrow. Due to the limited human cells in the DKO-hu mouse blood, it is not possible to functionally 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 deleterious effect on immune system and contribute to disease progression \(^{22,50}\). 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 pathogenesis in vivo, 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 \(^{32}\). 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 novel therapeutic intervention that targets pDC functions.
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Authorship statement: LZ, QJ and LS designed the project, analyzed data and wrote the paper; LZ, QJ, GL, JJ and GK performed experiments and analyzed data. The authors have no conflict of financial interest.
References


Figure Legend:

**Fig. 1. Development of pDC in lymphoid organs in DKO-hu mice.**

(A) Total live human cells (VLD- mCD45-hCD45+) from bone marrow (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+CD123+ pDCs of total human CD45+ cells. Human PBMC were used as control. (C) Summarized data show average percentage of pDCs (CD3- CD4+CD123+ of total human CD45+ cells) in different lymphoid organs from DKO-hu mice at 12 weeks post transplant (n=7). (D) Total numbers of pDCs in each lymphoid organ are calculated as described in material and methods. Error bars indicate standard deviations (n=7).

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

(A) pDCs (CD45‘CD3 CD4‘CD123‘) were purified as described in material and methods, and the purity was monitored by CD4 and CD123 staining (>95% pure). (B) 10,000 purified pDCs were cultured in the presence of influenza virus or HSV for 16 hours. Supernatants were collected and IFNα, IL-6, MIP1β and IP10 were measured by Human Cytokine Luminex kit. Error bars are standard deviations from triplicate samples. (C) Purified pDCs were stimulated with influenza virus or HSV for 48 hours, 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.

**Fig. 3. pDCs from DKO-hu mice express both HIV co-receptors, and are productively infected by HIV-1 in vivo.**

(A) Total bone marrow (BM) cells or splenocytes were analyzed by FACS. CD3‘CD4‘CD123‘ pDCs were further analyzed for CCR5 and CXCR4 expression. Dotted gray lines represent IgG isotype control. (B,C) At 8 days post HIV infection (R3A virus), splenocytes (B) and bone marrow cells (C) were stained with surface markers, followed by HIV p24 intracellular staining. The numbers in the plots represent p24+ percentage of CD3+ T cells and CD3’BDCA2‘CD123+ pDCs. Samples from one mock and 3 HIV-infected DKO-hu mice are shown. (D) Bone marrow cells from HIV-R3A-HSA infected DKO-hu mice were analyzed by flow cytometry. The
percentage of HSA (mouse CD24) expression on CD3^+CD8^- T cells and CD3^+CD123^+ pDCs from one mock and two HIV infected DKO-hu mice are shown.

**Fig. 4. HIV-1 infection induces pDC activation in DKO-hu mice.**

(A) Elevated IFNα in HIV-1 infected DKO-hu mice. Plasma from mock or HIV-1 infected mice at 1 week post infection was analyzed for IFNα by Human Cytokine Luminex kit. IFNα in the mock infected plasma is lower than the detection limit (<13 pg/ml). Standard deviation is shown as error bar (n=7 mice). (B) HIV-1 infection activated pDCs in the bone marrow in vivo. BDCA2^+CD123^+ pDCs from bone marrow 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 bone marrow (BM), spleen (SP) or mesenteric lymph node (mLN) cells. Error bars indicate standard deviations. 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 post HIV infection. p values between mock and HIV-infected groups were calculated by non parametric student t test. *, p<0.05, **, p<0.01, (G) pDC activation is correlated with CD4^- T cell activation and apoptosis. Bone marrow cells from 3 mock (open triangles) and 6 HIV infected (filled dots) mice were analyzed. Cells were stained with surface markers, followed by caspase3 intracellular staining. CD123^+BDCA2^+ pDCs and CD3^+CD4^- T cells from bone marrow were analyzed for Caspase3 or HLA-DR expression. Correlations were analyzed with Spearman’s nonparametric test, squared correlation coefficients (R^2) and p values were shown.

**Fig. 5. Differential depletion of CD4^- T cells and pDCs in HIV infected DKO-hu mice.**

(A) Human CD4^- T cells were depleted by HIV-1 infection. Total bone marrow cells from HIV infected DKO-hu mice were analyzed by FACS. The numbers in each plot represent the percentage of CD4^- T cells of total CD3^- T cells. (B) Human pDCs were not depleted. Total human CD45^- cells were analyzed for CD4^-CD123^- pDC. The number is percent pDCs of total human CD45^- cells. (C) Summarized data of human CD4^- T cells and pDCs in the bone marrow. (D) Summarized data of CD4^- T cells and pDCs in the spleen. Student's t test was used to calculate p values.
**Fig. 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 post infection was shown. (B) IFNα in the plasma of mock or HIV-infected DKO-hu mice was measured by Human Cytokine Luminex kit. Error bars are standard deviation (n=4). *, p < 0.05. (C/D) Bone marrow cells from mock or HIV infected DKO-hu mice were stimulated with HSV for 16 hours. The expression of intracellular IFNα in pDCs were measured by FACS (C). (D) Summarized data from 4 mock and 4 HIV infected DKO-hu mice are shown. (E/F) Bone marrow cells were also stimulated with Influenza virus and analyzed for the expression of IFNα in pDCs. (F) Summarized data from 4 mock and 4 HIV infected DKO-hu mice are shown. Error bars represent standard derivations. *, p< 0.05.
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