HIV-1 INFECTION AND PATHOGENESIS IN A NOVEL HUMANIZED MOUSE MODEL

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Running title: A novel HIV-1 pathogenesis mouse model

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Abstract

The Rag2-γC double knockout (DKO) mouse lacks T, B and NK cells, and allows development of a functional human immune system with human CD34+ hematopoietic stem/progenitor cells (DKO-hu HSC). Normal human T, B, and dendritic cells are present in peripheral blood, thymus, spleen and lymph nodes. We report that both CCR5 and CXCR4 are expressed on human immature and mature T cells. DKO-hu HSC mice allow efficient HIV-1 infection with plasma high viremia. High levels of productive infection occur in the thymus, spleen and lymph nodes. Human CD4+ T cells are gradually depleted by HIV-1 in a dose-dependent manner. In addition, HIV-1 infection persists in infected DKO-hu HSC mice for at least 19 weeks, with infectious HIV-1 in lymphoid tissues. Thus, the DKO-hu HSC mouse can serve as a relevant in vivo model to investigate mechanisms of HIV-1 infection and immuno-pathogenesis as well as to develop anti-HIV-1 therapeutics.
Introduction

The hallmarks of AIDS are high levels of HIV-1 infection, depletion of CD4+ T cells and loss of immunity. The mechanism of HIV-1 immuno-pathogenesis is not clear because human patients are the only host which supports high levels of HIV-1 replication and develops AIDS. Extensive studies have been performed in primate models with either low levels of HIV-1 replication (HIV-1 in chimps) or a different virus (SIV or SHIV in monkeys).\textsuperscript{1-3} HIV-1 fails to infect murine cells due to blocks at multiple steps of the HIV life cycle. The mouse with reconstituted human immune system would be an ideal small animal model for studying HIV-1 infection and pathogenesis. A number of human-mouse chimeric models have been developed, but with only limited success. The SCID-hu Thy/Liv mouse has an intact human thymus organ, thus allowing investigation of HIV-1 pathogenesis in a human lymphoid organ.\textsuperscript{4-6} However, very low levels of human T cells are detected in the peripheral lymphoid organs or blood. The hu-PBL-SCID mouse supports transient and selective engraftment of xeno-reactive human T cells.\textsuperscript{7,8} Human CD34+ cells transplanted into SCID or NOD/SCID mice lead to generation of mainly human myeloid and B cells in the mouse bone marrow, but inefficient generation of human T cells.\textsuperscript{9,10} When CD34+ human HSC/HSPC are injected directly into the liver of newborn Rag2-γC double knockout (DKO) mice, which lack T, B and NK cells, the newborn liver environment of DKO mice support efficient engraftment and development of a functional human immune system in central and peripheral lymphoid organs.\textsuperscript{11,12}

To demonstrate that the DKO-hu HSC mouse can support efficient HIV-1 infection with relevant immuno-pathogenesis, we show that CCR5 and CXCR4, the HIV-1 coreceptors, are both expressed on human T cells in central and peripheral lymphoid organs. DKO-hu HSC mice allow high plasma viremia with high levels of HIV-1 infection in lymphoid organs. Both CD4+CD45RO+ and CD4+CD45RO- T cells are productively infected in lymphoid organs. Human CD4+ T cells are gradually depleted by HIV-1 in a dose-dependent manner. In addition, HIV-1 infection persists in infected DKO-hu HSC mice for at least 19 weeks, with recoverable infectious HIV-1 in lymphoid tissues.

Study design

Construction of DKO-hu HSC mice with human CD34+ cells from cord blood or fetal liver:

Human CD34+ cells were isolated from cord blood or fetal liver tissues. 1x10^6 FL CD34+ or 1x10^5 CB CD34+ cells were injected intra-hepatically into each newborn DKO mouse previously
irradiated at 400 rad. Human leukocytes (CD45+) were analyzed for CD3, CD4, CD8, CD45RO, CD19, CCR5(2D7) and CXCR4(12G5) by FACS.

**HIV-1 infection and pathogenesis in DKO-hu HSC mice:**

At least 15 weeks post CD34 HSPC transfer, DKO-hu HSC mice with stable human leukocyte reconstitution were infected with X4/R5 dual tropic, R5-tropic or X4-tropic HIV-1 (NL4-R3A, JRCSF or NL4-3, respectively) intravenously (iv). Mock supernatant or heat-inactivated HIV-1 stocks were used as negative controls. Plasma viral load was monitored with the Roche Amplicor Monitor v.1.5 assay (Roche Diagnostics Corporation, Indianapolis, IN). Blood CD4+ T cells were measured by GUAVA Easycytes (GUAVA, Hayward, CA) and by FACS. Central and peripheral lymphoid organs were harvested to investigate HIV replication and pathogenesis in the thymus, spleen and lymph nodes. To detect infectious HIV-1 in lymphoid organs, splenocytes or thymocytes were co-cultured with PHA-activated PBMC for 14 days.

**Results and discussion**

We transplanted CD34+ HSPC derived from human cord blood or fetal liver into newborn DKO mice intra-hepatically. Between 8 and 33 weeks post transplant, we analyzed human leukocytes in the peripheral blood and lymphoid organs of these DKO-hu HSC mice. Greater than 90% of the transplanted DKO mice have stable engraftment with human CD45+ cells (Fig. 1A). CD3+CD4+ cells (10-20% of total PBL) were detected in transplanted mice at 14 weeks post transplant (Fig. 1A). Long-term development of normal T cell subsets (33 weeks post transplant) is also observed in transplanted mice (Fig. 1B), suggesting maintenance of human HSPC in DKO-hu HSC mice as previously reported. The HIV-1 entry coreceptors CCR5 and CXCR4 are both expressed on human CD4+ T cells (Fig. 1C). While the levels of CCR5 expressed on human CD4 T cells derived from DKO-hu HSC mice are comparable to that from primary human tissues or cells, a reduced expression of CXCR4 is detected on human CD4 T cells from DKO-hu HSC mice. As in human primary T cells, CD45RO+ T cells predominantly express CCR5, whereas CD45RO- T cells efficiently express CXCR4 (Fig. 1C).

To test HIV-1 infection of DKO-hu HSC mice in vivo, we first used the NL4-R3A recombinant virus with the R5/X4 dual tropic R3A Env. R3A was transmitted in the patient (IV drug user) with rapid disease progression, and infected both macrophages and T cells. When inoculated with high dose (20,000 IU/mouse) of the NL4-R3A virus, DKO-hu HSC mice showed very high plasma viral loads (200,000-400,000 copies/ml) at 1 week post infection (wpi) and 10,000
copies/ml at 2-4 wpi. Interestingly, HIV-1 plasma viremia was still detectable (947 copies/ml) with infectious HIV-1 at 12 wpi (Fig. 2A and data not shown). The mock-infected DKO-hu HSC mice or HIV-infected control DKO mice with no human cells showed no detectable HIV-1 genome (Fig. 2A and Table 1). In the infected DKO-hu HSC mice, CD4+ T cell numbers in the blood were significantly depleted at 2, 3, 4 and 12 weeks post infection (Fig. 2B).

When infected with a lower dose of NL4-R3A (4,000 IU or 1 ng/mouse), HIV-1 replication peaked at 2-3 wpi, correlated with depletion of CD4+ T cells (Fig. 2C). Viremia decreased to low or undetectable levels in the infected DKO-hu HSC mouse, correlated with an increase of CD4+ T cells (Fig. 2C). At 19 wpi with no detectable HIV-1 viremia (<500 copies/ml), infectious HIV-1 was recovered from thymocytes, but not from splenocytes or PBMC in the mouse (Fig. 2G). In the lymphoid organs at 1 wpi, high levels of productive infection were detected in the thymus, spleen and LN (Fig. 2D&2E). Interestingly, both CD45RO+ and CD45RO- T cells are infected (Fig. 2D) as in HIV-infected lymphoid organs.23 The CD4+ T cells are also preferentially depleted in the thymus and LN (Fig. 2F and Table 1). Efficient HIV-1 infection is also detected with R5-tropic JRCSF and X4-tropic NL4-3 in DKO-hu HSC mice (Table 1).

The DKO-hu HSC mouse will serve as a relevant HIV-1 infection and pathogenesis model. First, the mouse supports efficient and stable development of human T, B and myeloid cells in central and peripheral lymphoid organs.11,12 Second, HIV-1 infection leads to high and persistent HIV-1 viremia in PB and in lymphoid organs (this report and a recent report24). Third, human CD4+ T cells are depleted by HIV-1 in a dose- and virus-dependent fashion. However, the human antibody or T cell response is weak in the DKO-hu HSC mouse (L. Zhang and L. Su, unpublished results and24). In a recent report, NOD/SCID/IL2Rγ null mice humanized by cord blood-derived CD34+ cells have been shown to support HIV-1 infection with both CCR5- and CXCR4-tropic HIV-1 isolates for more than 40 days.25 Anti-HIV-1 antibodies are detected only in animals with high levels of viral infection. The DKO-hu HSC mouse, and its improved derivatives, will facilitate development of novel therapeutic strategies for controlling HIV-1 diseases.

Acknowledgments

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References

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Figure legend

Figure 1. Human T cells express both CCR5 and CXCR4 in DKO-hu HSC Mice.
Human CD34+ cells were injected intra-hepatically in newborn DKO mice. **A:** A representative cohort of 12 DKO-hu HSC mice reconstituted with FL CD34+ cells is analyzed by FACS. Individual open bars represent percentage of human CD45+ cells in total peripheral blood lymphoid cells (14 weeks post transplant). The dark bars indicate the percentage of human CD3+CD4+ cells. **B:** Long-term T cell reconstitution in DKO-hu HSC mice. At 33 weeks post transplant, the CD4+/CD8+ pattern on human CD45+ cells in the PBMC, spleen, and thymus are analyzed. **C:** Expression of HIV-1 Co-receptors on human CD4+ T cells. Representative FACS analysis of CCR5 (top) or CXCR4 (bottom) on human CD45+CD3+CD4+ cells in PBMC and lymphoid organs of DKO-hu HSC mice. ITTP, intrathymic T progenitor (CD4+CD8-CD3+); DP, double positive (CD4+CD8+ thymocytes); CD4SP, CD4+ single positive (CD4+CD8-thymocytes).

Figure 2. HIV-1 Replication and Pathogenesis in DKO-hu HSC mice.
Intravenous infection of HIV-R3A at high (A-B) and low (C-G) doses was performed in DKO-hu HSC mice. **A:** NL4-R3A stock (5 ng p24 or 20,000 IU/mouse) was used to infect DKO-hu HSC mice (HIV DKO-HSC, solid diamond and square) and DKO mice (HIV DKO, crosses). Two DKO-hu HSC mice were also mock infected as controls (mock DKO-HSC, open diamond and square). Plasma samples were collected at 1, 2, 3, 4 and 12 weeks post infection and HIV genome copy numbers were determined. The sensitivity of detection is 500 copies/ml due to necessary sample dilution. **B:** Human CD4+ T cell number in the blood of DKO-hu HSC mice was determined at 15 weeks post HSPC transfer (1 week prior to HIV infection, -1 wpi), and determined again at 1, 2, 3, 4 and 12 weeks post infection. **C:** DKO-hu HSC mice were infected with low dose NL4-R3A (1ng p24 or 4,000 IU/mouse). Plasma HIV genome (triangle, left Y axis) and CD4 cell counts (diamond, right Y axis) in mouse #3 (Table 1) are shown. **D:** FACS analysis of CD45+ thymocytes by co-staining p24 with CD4 or of CD45+CD4+ lymph node cells with CD45RO from mice infected with NL4-R3A (1 wpi). **E:** Immuno-histochemistry was performed with anti-p24 antibody on paraffin sections of lymphoid tissues. Lymph Node (i), thymus (ii), spleen (iii) and the H&E staining of adjacent section of the spleen (iv). Arrows in (iii) and (iv) indicate the CD45+ follicles in the spleen. Original magnification is 4x for all images. **F:** Depletion of CD4+ T cells in lymphoid organs. At 1 wpi, human CD4 and CD8 in the thymus and lymph nodes of mock or NL4-R3A infected mice were analyzed by FACS. The percentage of each population is indicated. **G:** Thymocytes (4,500 CD45+ cells) or splenocytes
(17,000 CD45+ cells) from mouse #4 (Table 1) harvested at 19 wpi were co-cultured with PHA-activated PBMC to detect infectious HIV-1 (p24/ml). Similar results were observed with cells from mouse #3 at 14 wpi.
Table 1: Summary of HIV-1 infection in the DKO-HSC mice

<table>
<thead>
<tr>
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<th>CD34+ Cells</th>
<th>WPT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HIV RNA per ml blood/&lt;sup&gt;(CD4/CD8 Ratio&lt;sup&gt;c&lt;/sup&gt;)&lt;/sup&gt;</th>
<th>Weeks Post Infection</th>
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<td>HIV RNA (ng/mouse)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1</td>
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<tr>
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<td>CB</td>
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<td>Mock</td>
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<sup>a</sup>: “WPT”, weeks post transplant, indicates initiation of HIV-1 infection.

<sup>b</sup>: The NL4-R3A and NL4 were titered on Hela-CD4-LTR-β-gal cell line. For NL4-R3A, 1 ng p24 equals 4000 infection units. For NL4-3, 1 ng p24 equals 500 infection units. JRCSF was titered on U373-MAGI-CCR5E cells, 1 ng p24 equals 20 infection units. (Both cell lines obtained from NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID).

<sup>c</sup>: Percentage of CD4+ cells divided by percentage of CD8+ in the blood.

<sup>d</sup>: Mouse 3# sample is from 5 weeks post infection.

<sup>e</sup>: “nd” Non-detectable, below the detection level (<500 copies/ml).

*: No human CD45+ cells detected in the blood.
Zhang et al. Figure 1
Zhang et al. Figure 2
HIV-1 infection and pathogenesis in a novel humanized mouse model

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