PRODUCTIVE HIV-1 INFECTION IN CRISPR CD4/CCR5/C1qbp KNOCK-IN CD8-DEPLETED MICE

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BACKGROUND

Rodents cannot be infected with HIV due to multiple obstacles. First, HIV requires human CD4 receptor and CCR5/CXCR4 co-receptor on human immunocytes, each are structured differently in mice. Second, there are post-transcriptional blocks for viral replication in rodents. These include excessive viral RNA splicing limiting genomic RNA for viral assembly and release. Third, post-transcription factors absent in rodent cells limits viral RNA export, translation, stability, and packaging. Fourth, rodent CD8+ T viral control is active in rodents with active killing virus-infected cells and antiviral cytokine release. Attempts by others to use transgenic mouse models have all failed due to random transgene insertion and expression, and disruption of endogenous genes. These led to off-target toxicities and blocks viral assembly. Herein, a knock-in (KI) of huCD4, huCCR5, and huC1qbp and CD8+ T cell control resulted in stable physiologically HIV expression.

METHODS

CRISPR-Cas9 homologous recombination inserted human CD4, CCR5, and C1qbp[D106G] into defined loci in the mouse genome generating physiologically relevant human expression patterns. Mouse C1qbp (also known as C1QBP or p32) which blocks viral growth was replaced with specific human amino acid. All site-specific CRISPR KIs were confirmed using RT-qPCR and Sanger Sequencing. CD4+ T-cells isolated from peripheral blood lymphocytes, splenocytes, thymocytes, and lymph nodes expressed human CD4 and CCR5 cell receptors in the correct orientation.

RESULTS

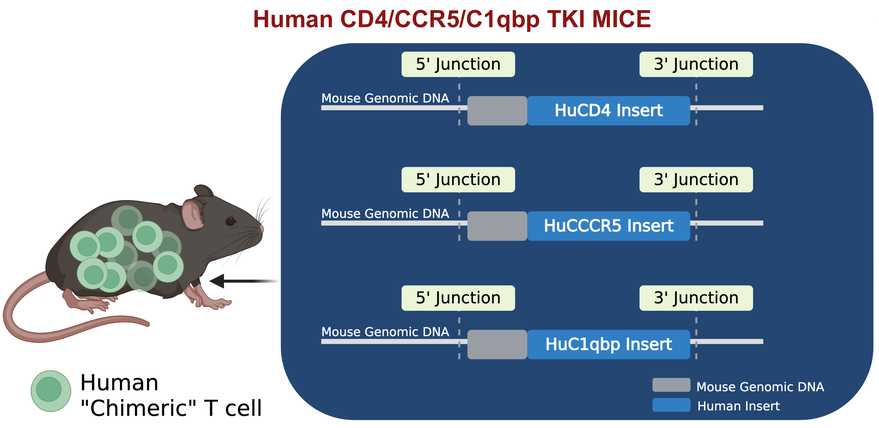


Figure 1. Schematic of the CRISPR-mediated strategy for generating a polygenic "humanized" mouse model via targeted knock-in (KI) at orthologous loci. Full-length human coding sequences (hCDS) for CD4, CCR5, and C1qbp were precisely integrated to replace endogenous mouse sequences, resulting in simultaneous host gene knockouts (KO) in embryonic stem (ES) cells. Each human transgene is flanked by 5' and 3' homology arms (HA) and remains under the transcriptional control of native mouse regulatory elements to preserve physiological expression profiles. This approach facilitates the development of "chimeric" murine cells, such as T cells.

A CRISPR-engineered mouse model overcomes natural species barriers, enabling productive HIV infection, providing a vital platform for testing future vaccines and therapeutics.

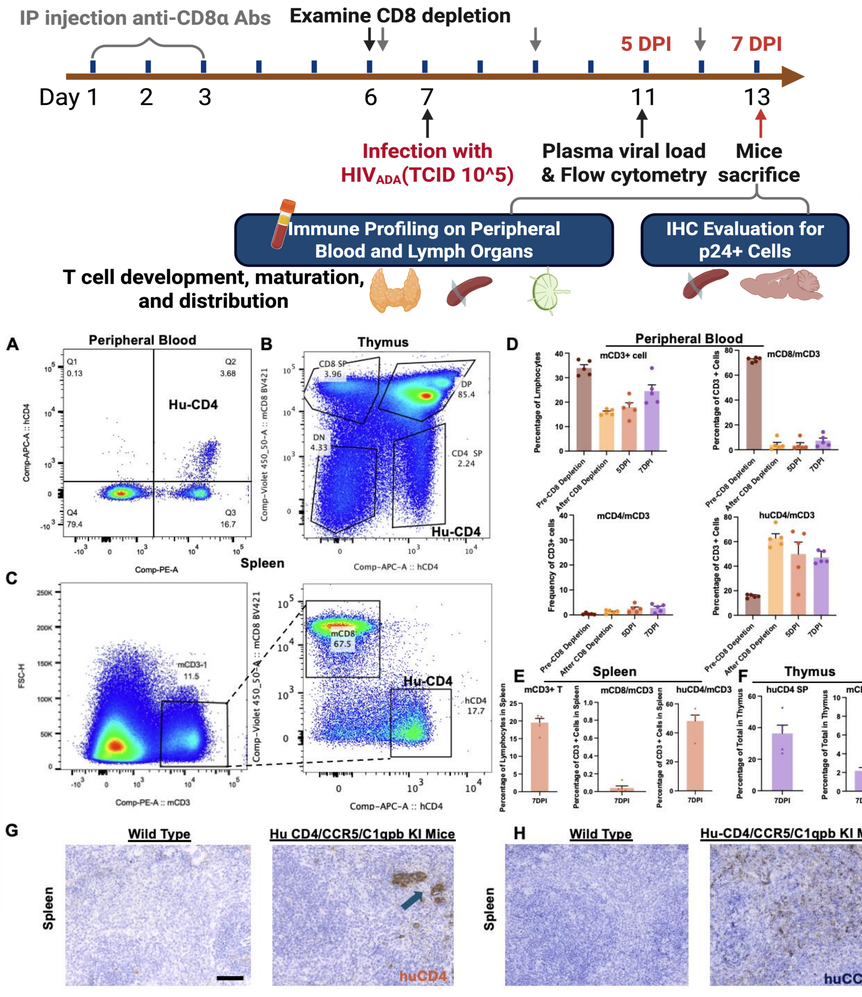


Figure 2. Selection expression of human CD4 and CCR5 on T cells in huCD4/CCR5/C1qbp KI mice. (A–C) Flow-cytometric analysis of single cells isolated from peripheral blood **(A),** thymus **(B),** and spleen **(C)** of huCD4/CCR5/C1qbp KI mice. Cells were stained with anti-human CD4, anti-mouse CD3, anti-mouse CD4, and anti-mouse CD8 antibodies. Representative dot plots show huCD4 expression on mCD3$^+$ lymphocytes and the distribution of thymic DN, DP, CD4 SP and CD8 SP subsets **(B)** as well as splenic mCD3$^+$, mCD8$^+$ and huCD4$^+$ T cells **(C).** The percentage of cells in each gate is indicated on the plots. **(D–F)** Quantification of T-cell subsets in peripheral blood **(D)**, spleen **(E),** and thymus **(F). (G-H)** Representative images of human CD4$^+$ and CCR5+ cells in spleen sections at 20× magnification. Blue arrows indicate individual or clustered human CD4$^+$ cells.

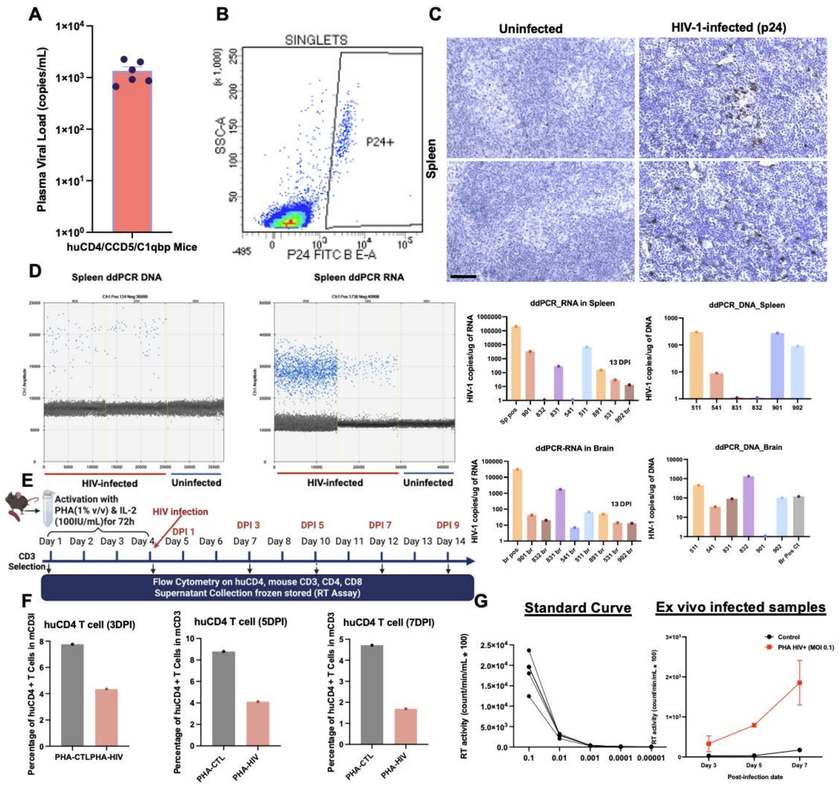


Figure 3. HIV-1 infection in huCD4/CCR5/C1qbp mice and primary splenocytes. (A) Plasma viral load, **(B)** Representative HIV-1 p24 flow cytometry, **(C)** immunohistochemistry antigen evaluation and **(D)** Droplet Digital PCR (ddPCR) quantification of HIV-1 RNA/DNA copies in spleen and brain five days after HIV-1$_{ADA}$ challenge. **(E)** Experimental schematic of the ex vivo HIV-1 challenge study. Primary splenocytes was isolated and prepared in single cell suspensions, followed by CD3 enrichment using EasySep™ Mouse T Cell Isolation Kit and activated with PHA/IL-2 for 72h prior to infection with HIV-1$_{ADA}$(MOI = 0.1) **(F)** Frequency of huCD4+ T cells at 3, 5 and 7 dpi, comparing PHA-stimulated control using flow cytometry **(G)** Kinetic analysis of Reverse Transcriptase (RT) activity in culture supernatants over 7 days, including a standard curve (left) and ex vivo infected samples (right), indicating productive viral replication.

CONCLUSIONS

- Several viral replication blocks have been reported in mice. These include barriers to viral entry, transcription, and assembly. The CRISPR KI mouse model offers physiologically regulated expression of HIV entry receptors and represents a notable advancement over any previous transgenic system.
- The mouse provides a new platform for studying HIV entry, early replication, and virion production to study human therapeutic and vaccine strategies.
- The next-generation mouse model with the addition of human MHC class II in huCD4/CCR5/C1qbp mouse model that are known to be essential for the maturation and activation of huCD4+ T-cells is being bred.

FUNDING SOURCES

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