IMMUNE CONTROL OF LIVE ATTENUATED-HIV INFECTION AND DISEASE IN BLTS-HUMANIZED MICE

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BACKGROUND

Humanized mouse models are important tools for studying HIV/AIDS; however, several limitations have been reported in current models, including poor secondary lymphoid tissue development and immune-mediated control of attenuated viruses. The recently developed Bone marrow-derived human hematopoietic stem cells (hHSCs), Liver, Thymus and Spleen (BLTS) humanized mouse model supports robust development of human lymphoid and myeloid cells. We hypothesize that BLTS-humanized mice, having a more complete repertoire of innate and adaptive immune cells, will recapitulate the human anti-HIV immunity, and be particularly effective at controlling live-attenuated HIV including the Nef deleted (ΔNef)/mutated strain of HIV.

METHODS

- BLTS humanized mice were generated by co-engrafting immunodeficient mice with autologous human hematopoietic stem cells via intravascular injection, and with human lymphoid tissues for hematopoiesis (fetal thymus, liver and spleen) via kidney capsule transplant.
- Reconstitution and characterization of human immune cells was determined by flow cytometry. Humanized animals were inoculated with different HIV strains including WT, Nef deleted (ΔNef) or Nef mutated virus.
- Single amino acid mutations were done in dimerization domain of HIV Nef, which is essential for Nef function and pathogenicity of the virus.
- Viral load was measured by qPCR and lymphoid tissue pathology was examined via immunohistochemistry.

RESULTS

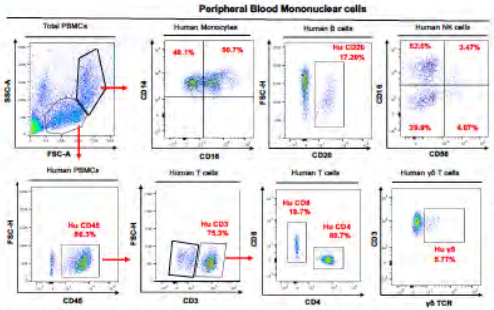

Fig.1. Reconstitution of human immune cells (Monocytes B cells, T cells, γδ T cells and NK cells) in humanized BLTS mouse.

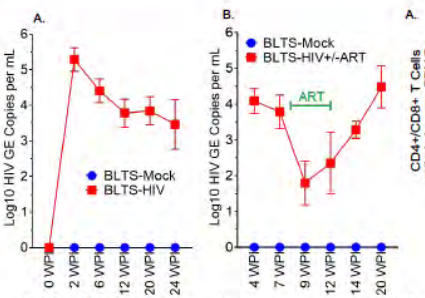

Fig.3. A) BLTS humanized mice support HIV infection B) ART mediated suppression of the HIV virus replication.

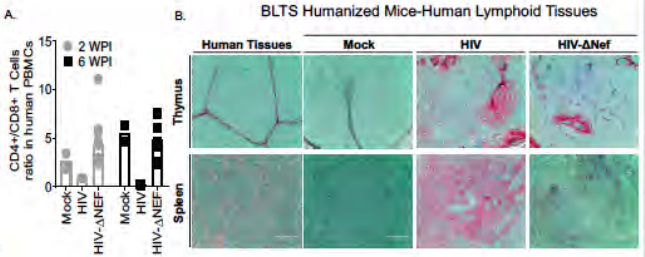

Fig.5. A) Human CD4+/CD8+ T cells ratio is inverted in HIV infected BLTS mice but not in mock or Δ Nef infected mice. B) Lymphoid tissue from HIV WT infected mice show more fibrosis than Δ Nef infected mice.

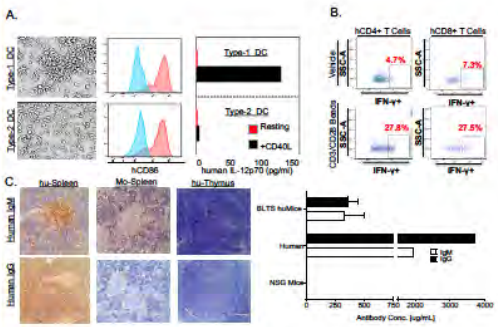

Fig.2. Human immune cells reconstituted in humanized BLTS mice are functionally active. A. DCs generated from humanized mice stem cells produce IL-12p70 B) T cells isolated from BLTS mice produce IFN-γ in response to anti CD3-anti CD28 stimulation. C) B cells isolated from BLTS mice secrete antibodies.

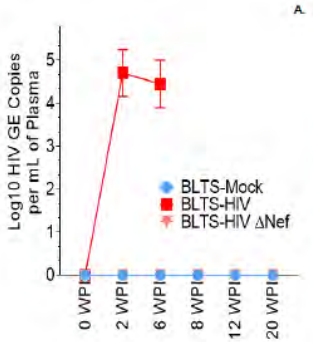

Fig.4. A) BLTS humanized mice infected with HIV ΔNef limits virus replication whereas high virus titer is detected in WT HIV infected BLTS mice .

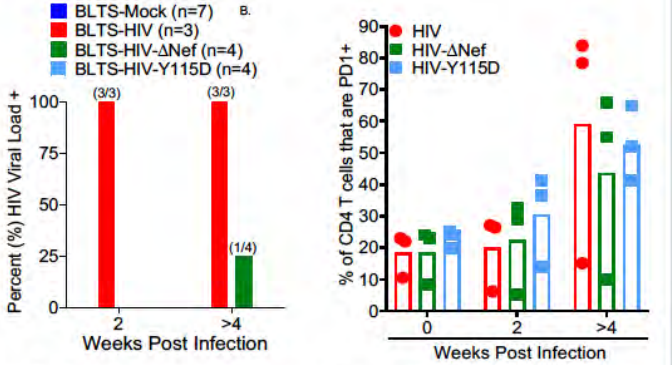

Fig.6. A) Complete deletion or single amino acid mutation (Y115D) in HIV Nef protein controls/limits the HIV replication B) CD4 T cells from BLTS mice infected with Nef mutated HIV are less exhausted than CD4 T cells from WT infected mice.

CONCLUSIONS

- ❖The BLTS-humanized mice exhibit a robust development of human myeloid and lymphoid cells, including functionally active T Cells (αβ and γδ T cells), NK cells, antibody-secreting B cells and dendritic cells.
- ❖BLTS mouse model also supports HIV infection and HAART-mediated HIV suppression.
- ❖Unlike other humanized mouse models, infection of BLTS humanized mice with live-attenuated ΔNef HIV results in long-term aviremia.
- ❖Unlike WT HIV infected mice, CD4 T cell are not depleted in ΔNef HIV infected BLTS mice.
- ❖BLTS mice mount effective antiviral immune responses with reduced lymphoid tissue pathology when infected with ΔNef HIV.
- ❖BLTS mice infected with Nef mutant (Y115D) control infection, highlighting this mutation site as a potential therapeutic vaccine target.