HIV-1 Infection and Alzheimer's Disease Pathobiology in a Novel Humanized APP-Knock in Mouse Model

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Introduction

- Antiretroviral therapy (ART) has increased the lifespan of people living with HIV (PLWH).
- Nearly 50% of PLWH develop cognitive impairments, and the incidence of Alzheimer's disease (AD) and associated dementia is higher in PLWH.
- The interactions between viral infection, low levels of CD4+ T cells, aggregation of misfolded proteins, and secondary effects of ART may exacerbate AD in PLWH.
- Low CNS penetrations of ART allow persistent viral infection in the brain.
- We hypothesize that HIV-1-induced neuroinflammation drives AD progression.
- Species restrictions of HIV-1 infection prevent simultaneous study of AD in the context of HIV-1 infection.

Background

- A novel humanized AD mouse model was created by knocking in human APP^{KM670,671NL}(APP-KI), PS1^{M146V}, or MAPT^{P301S} using CRISPR technology in an immunocompromised NOG background.
- These advances eliminate the expression of endogenous murine homologs.
- APP-KI mice are crossed with NOD/Shi-*scid*/IL-2Rγ^{null}Tg(CMV-IL34) (NOG/hIL34) mice that are permissive for to generate APP-KI/hIL34 (**NAIL34**):
  - Human adaptive and innate immune reconstitution
  - Development of human microglia in the brain
  - Productive HIV infection in the brain

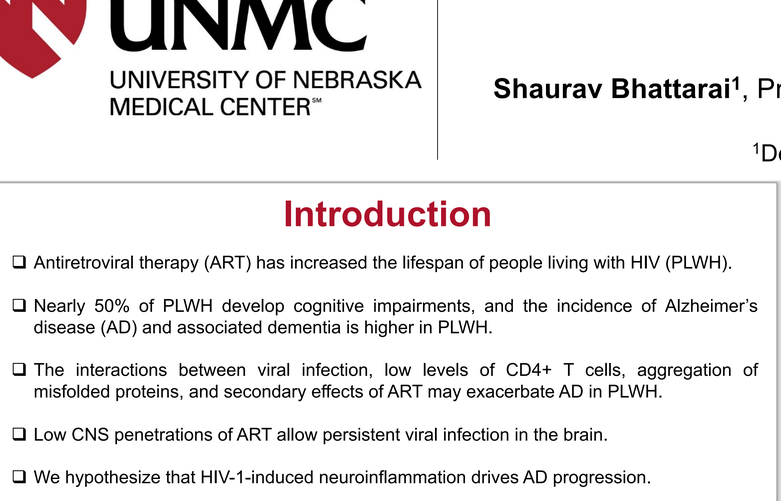
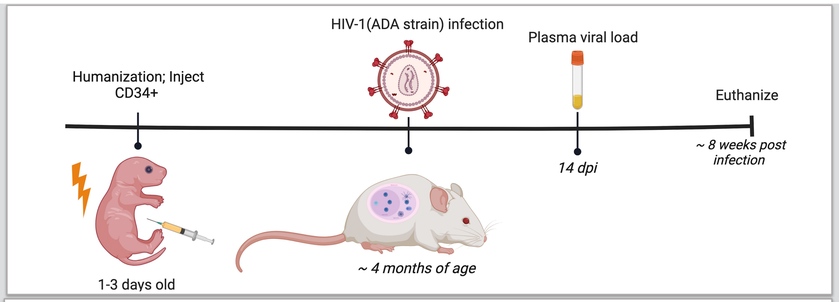


Fig. 1. Generation of AD knock-in mouse models: (**A**)APP$^{KM670/671N}$, (**B**) MAPT^{P301S} and, (**C**) PS1^{M146V} mice, changes in nucleotides and corresponding amino acids and sanger sequencing confirming replacement of pathological (red box) and humanized (green box) nucleotides in APP16, MAPT and PS1 mouse. (**D**) Western blot analysis of human APP fragments of APP-KI mice showing full-length APP(22C11), human APP(6E10), APP-CTFβ, and APP-CTF α. (**E**) IHC staining for amyloid beta (Aβ) in 6-month-old mice showing intraneuronal Aβ inclusions.

Methods

- APP-KI/hIL34 (**NAIL34**) and NOG/hIL34 (hIL34) mice were reconstituted with CD34+ hematopoietic progenitor cells from cord blood to generate humanized APP-KI mice with functional adaptive and innate immune systems.
- Four-month-old, immune-reconstituted hNAIL and hIL34 mice were infected with macrophage-tropic HIV-1$_{ADA}$ (10^5 $TCID_{50}$).
- Plasma viral load and human immune cell profiling were done two weeks post-infection, and mice were sacrificed at eight weeks post-infection for the evaluation of HIV neuropathogenesis and AD-associated pathology.



Results

Development of human microglia and productive HIV-1 infection in humanized APP knock-in mice increases amyloid load

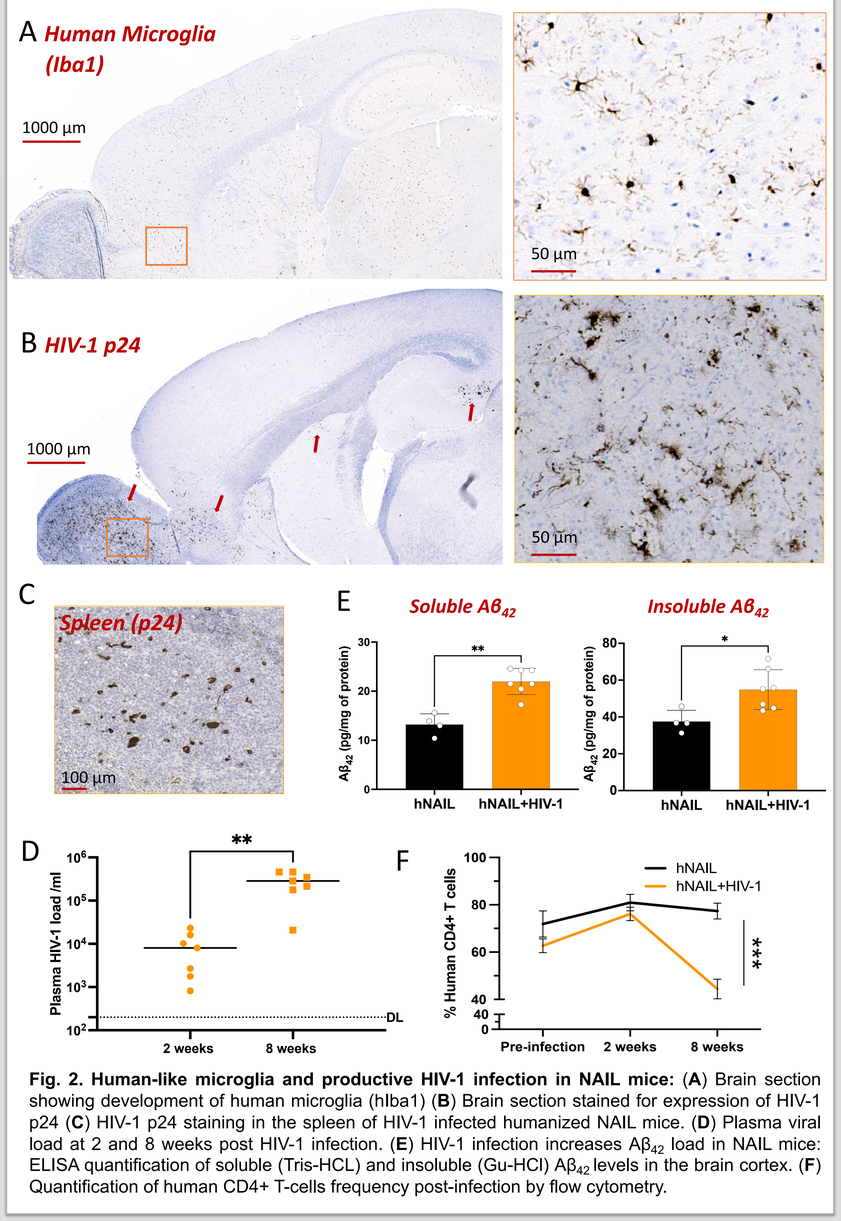


Fig. 2. Human-like microglia and productive HIV-1 infection in NAIL mice: (**A**) Brain section showing development of human microglia (hIba1) (**B**) Brain section stained for expression of HIV-1 p24 (**C**) HIV-1 p24 staining in the spleen of HIV-1 infected humanized NAIL mice. (**D**) Plasma viral load at 2 and 8 weeks post HIV-1 infection. (**E**) HIV-1 infection increases $Aβ_{42}$ load in NAIL mice: ELISA quantification of soluble (Tris-HCL) and insoluble (Gu-HCl) $Aβ_{42}$ levels in the brain cortex. (**F**) Quantification of human CD4+ T-cells frequency post-infection by flow cytometry.

Spatial transcriptomics elucidating HIV-mediated neuroinflammation

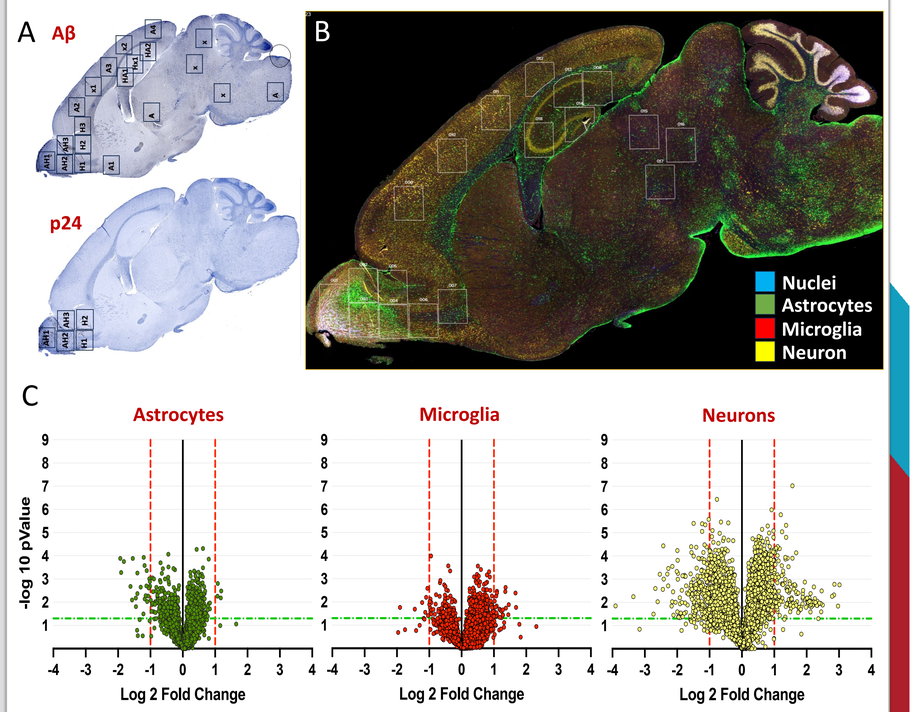


Fig.3. Spatial transcriptomics. (**A**) IHC staining of adjacent 5µm brain sections to identify regions of Aβ deposition and HIV-1 infection (p-24). (**B**) The middle 5µm brain section was stained for astrocytes (GFAP, green), microglia (IBA1, red), and neurons (NueN, yellow) and nucleus (DAPI, blue), to isolate cell-specific transcripts from regions with Aβ deposition and/or HIV-1 infection determined from IHC staining of adjacent brain sections (**A**). (**C**) Volcano plots of transcriptomic changes isolated from different cell types compared between brain regions with both Aβ-plaque+HIV-1 vs regions with Aβ-plaque alone.

Conclusions

- We generated novel AD mice (NAIL) by crossing APP-KI mice with NOG/hIL34, which allows for the simultaneous study of AD in the context of progressive HIV-1 infection.
- Reconstitution of the mice with CD34+ hematopoietic progenitor cells developed a human immune system in the periphery and human microglia in the brain.
- Humanized NAIL mice are susceptible to HIV-1 infection both in the periphery and the brain.
- HIV-1 infection of NAIL mice significantly increased amyloid burden compared to uninfected controls.
- Spatial transcriptomics is being performed to further elucidate the HIV-mediated neuroinflammatory transcriptomic signature from astrocytes, microglia, and neurons.
- This model, for the first time, will help study AD progression in the context of HIV and help develop disease-modifying therapies for HIV-associated AD disease and related dementia.

References

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Acknowledgements

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