Sharpening Our Tools: Developing Next-Generation Humanized Mouse Models for HIV and TB Research

Jack (Xiaozhi) Yang[1], Madeleine Lepard[1], Sam Afkhami[1], Anna Zganiacz[1], Aisha Nazli[1], Fatemah Vahedi[1], Alexandre Deshiere[2], Michel Tremblay[2], Ali Ashkar[1], Zhou Xing[1], Charu Kaushic[1], and Amy Gillgrass[1]

[1]McMaster Immunology Research Centre, McMaster University, Hamilton ON, Canada
[2]Research Centre of the University Hospital Centre of Quebec, Laval University, Quebec City QC, Canada

Michael G. DeGroote INSTITUTE FOR INFECTIOUS DISEASE RESEARCH

McMaster University

Immunology RESEARCH CENTRE

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

31st Annual Canadian Conference on HIV/AIDS Research

CONFERENCE CAHR 2022

Conflict of Interest Disclosure: Authors have no conflicts of interest

Email: yangx65@mcmaster.ca

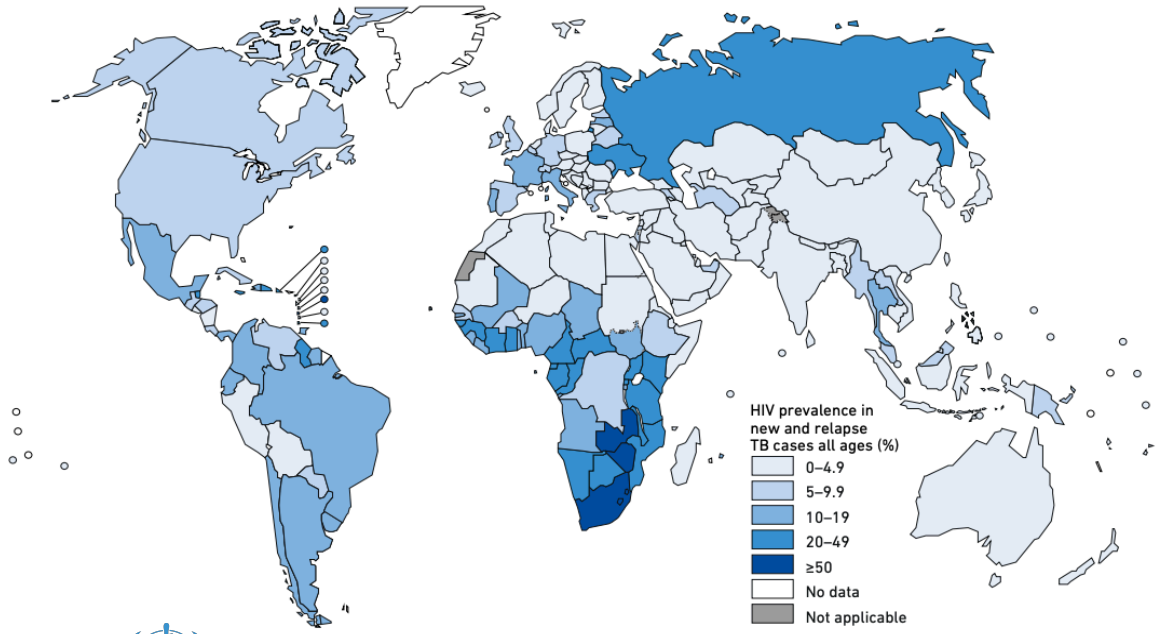
Background & Introduction

- Globally, there are 37.7 million people living with HIV (PLWH) and co-infection with Mycobacterium tuberculosis (Mtb) is the leading cause of death among PLWH.
- Nearly 2 billion people are infected with Mtb globally. Most are latently infected (with no clinical symptoms) but have a 5-10% chance of developing the contagious and deadly active pulmonary tuberculosis (TB).
- The risk for PLWH in developing active TB is increased by 20-fold. Unfortunately, geographic overlap of the high incidence of TB and HIV leads to high co-infection rates in areas such as sub-Saharan Africa.

Animal Models are critical to improve research progress in HIV/TB Co-infection:

- Widely Used models for in vivo HIV and TB studies:
  - Mouse, guinea pig, non-human primate
- **Issues:**
  - **Large animals**: Feasibility for widespread use (cost, ethics, sample size, etc.)
  - **Small animals**: Do not recapitulate certain features of TB granuloma pathology. HIV requires **human** cells for successful infection & thus will not infect standard mice.
- **Humanized Mice:**
  - Small animal (ease of maintenance)
  - Develop **human** CD4+ T cells & Macrophages
  - Form lung pathologies similar to humans

Estimated HIV prevalence in new and relapse TB cases (2017)



World Health Organization

Global tuberculosis report 2018. Geneva: World Health Organization

Methods & Experimental Design

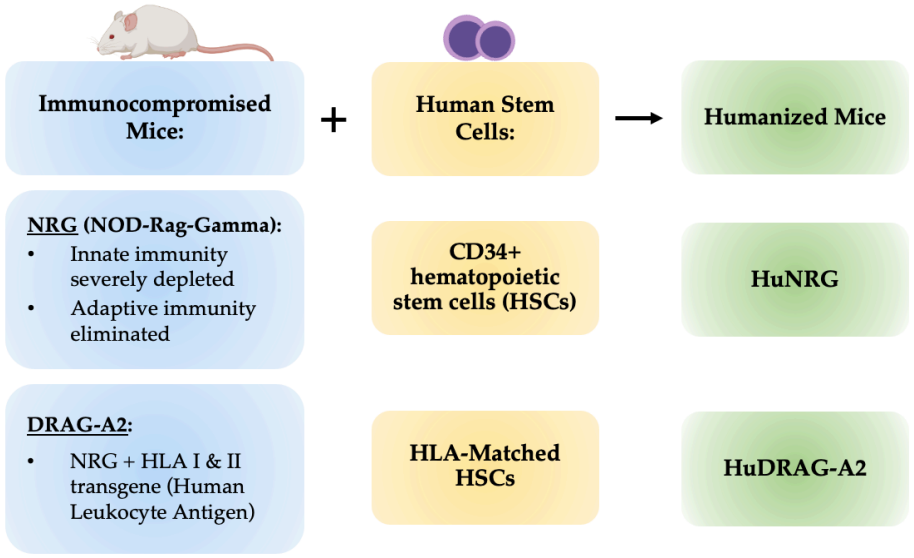


Figure 1. Humanized mice in our studies are generated by engrafting newborn (1-3 days) immunocompromised pups with CD34+ hematopoietic stem cells via intrahepatic injection.

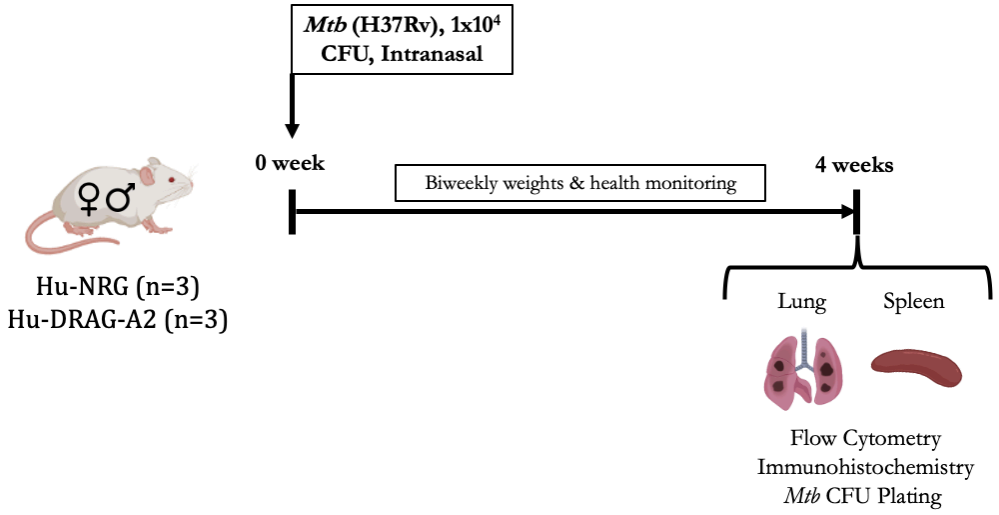


Figure 3. Establishing TB infection-alone within both the HuNRG and HuDRAG-A2 models.

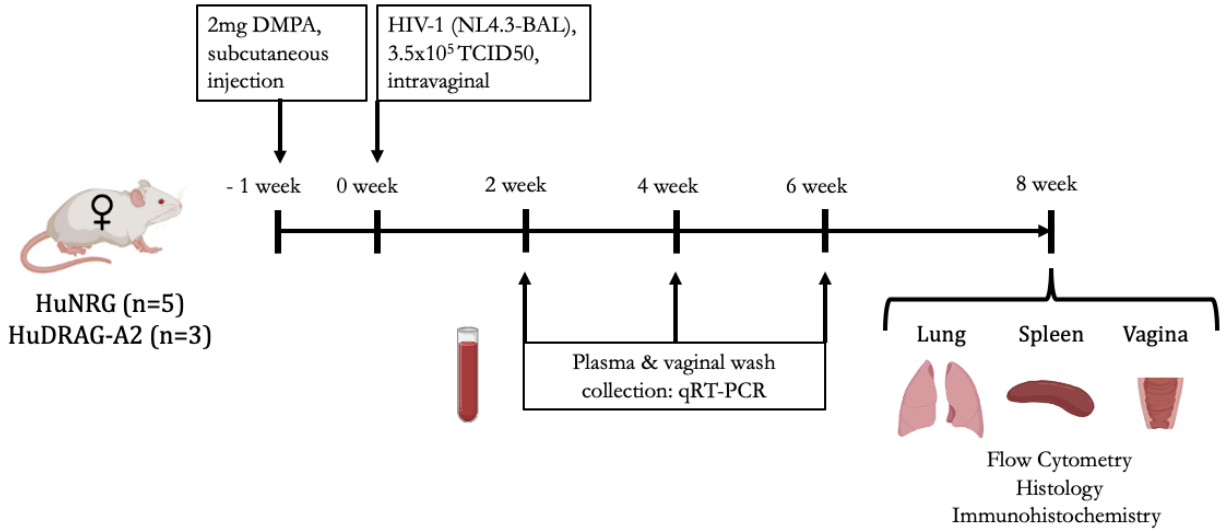


Figure 2. Establishing HIV Infection-alone within the HuNRG & HuDRAG-A2 models.

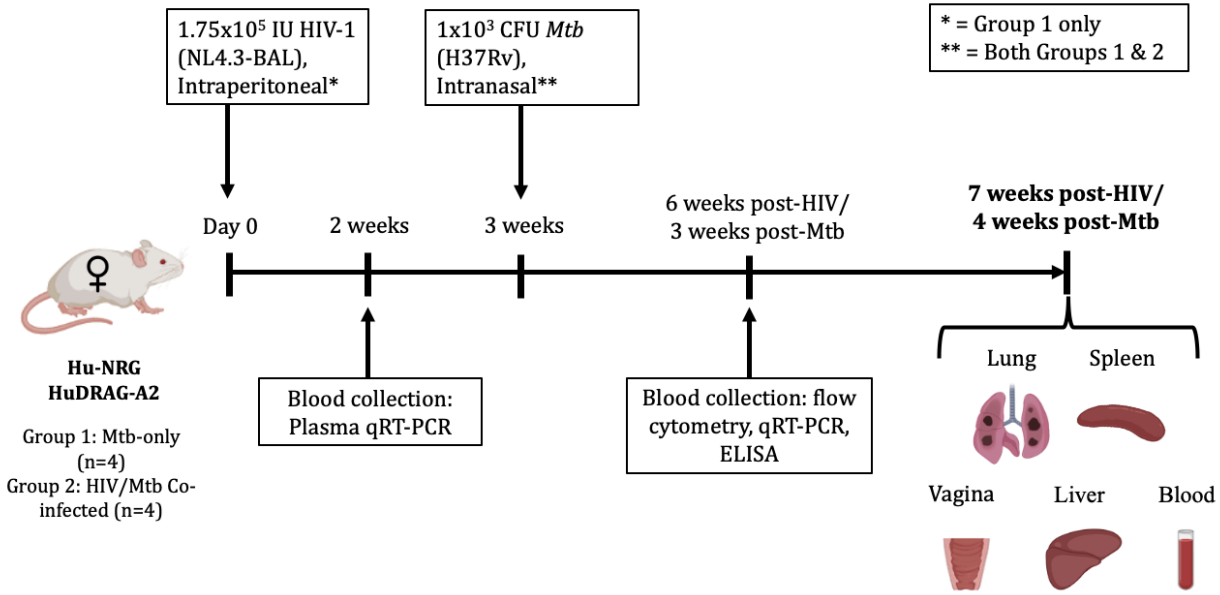


Figure 4. Proposed experimental methods for establishing HIV/TB co-infection within the HuNRG & HuDRAG-A2 model.

Results

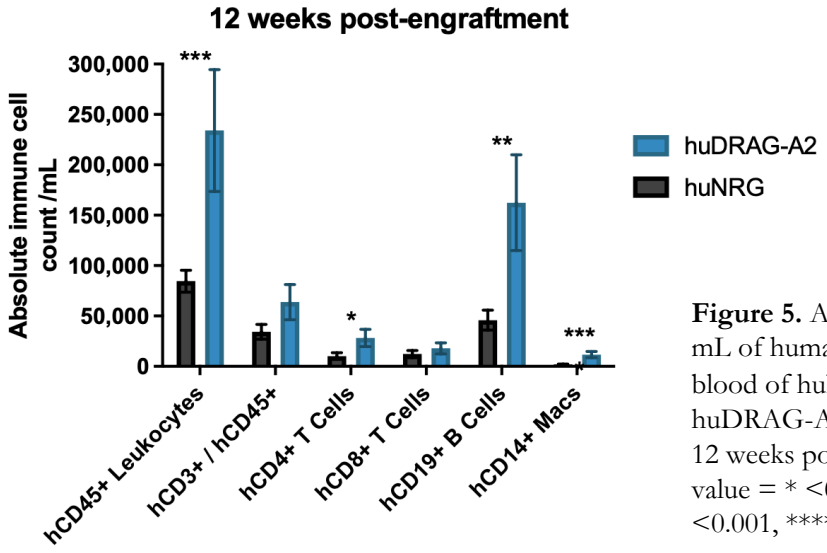


Figure 5. Absolute count per mL of human immune cells in blood of huNRG (N = 18) and huDRAG-A2 (N = 6) mice at 12 weeks post-engraftment. p value = * <0.05, ** <0.01, *** <0.001, **** <0.0001.

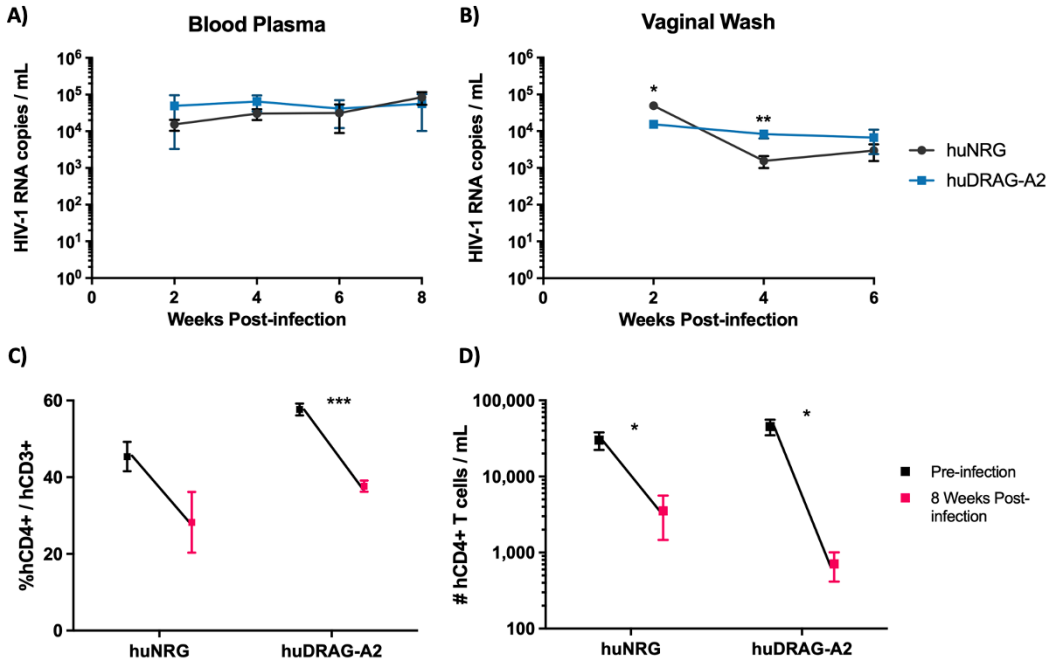


Figure 6. Viral load in the **(A)** blood plasma and **(B)** vaginal wash of huNRG (N = 5) and huDRAG-A2 (N = 3) mice post-HIV infection; **(C)** Percent and **(D)** absolute human CD4+ T cell frequency in the blood pre-infection compared to 8 weeks post-infection. p value = * <0.05, ** <0.01, *** <0.001.

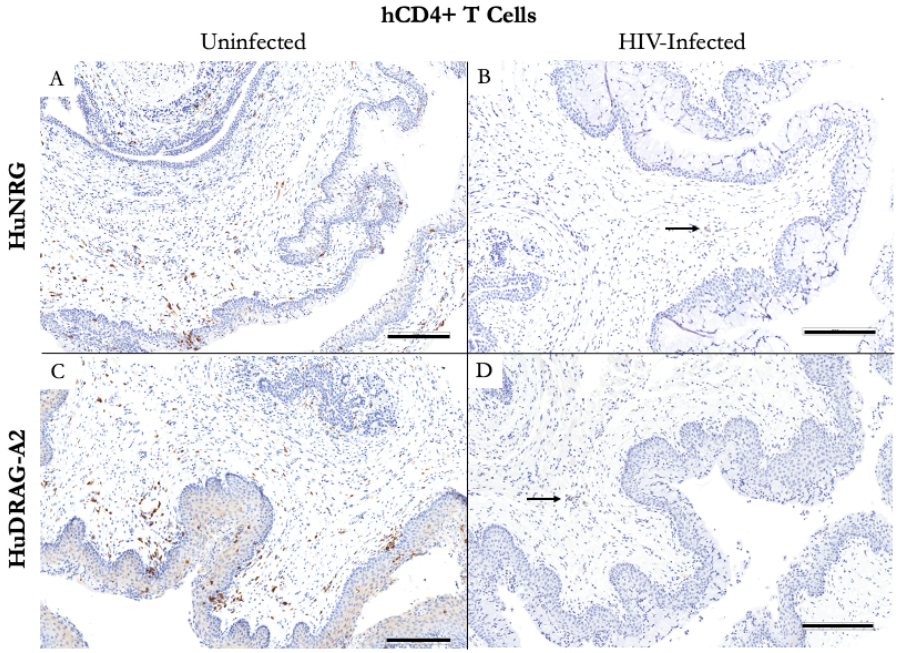


Figure 7. Human CD4+ T cell IHC of vaginal tissue of **(A)** Uninfected huNRG, and **(B)** HIV-infected huNRG at 8 weeks post-infection; **(C)** Uninfected huDRAG-A2, and **(D)** HIV-infected huDRAG-A2 at 8 weeks post-infection. (all images are at 10x, black scale bars = 200µm).

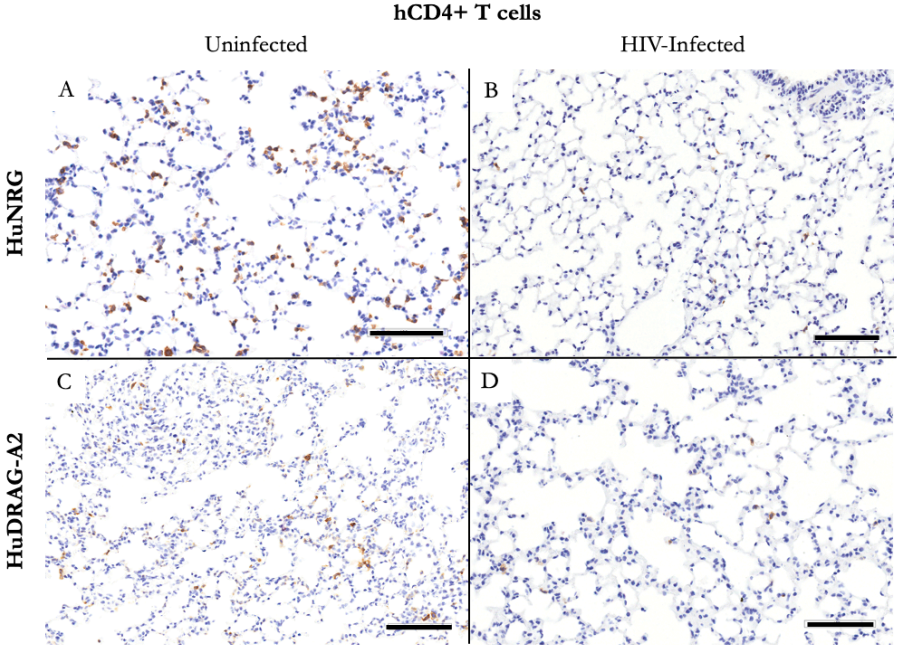


Figure 8. Human CD4+ IHC of lung tissue of **(A)** Uninfected huNRG lung **(B)** HIV-infected huNRG at 8 weeks post-infection; **(C)** Uninfected huDRAG-A2, and **(D)** HIV-infected huDRAG-A2 at 8 weeks post-infection. (all images are at 20x, black scale bars = 100µm).

Results

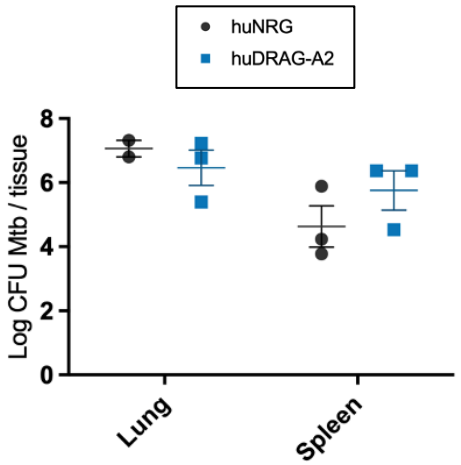


Figure 9. Mtb bacterial load in the lung and spleen of huNRG (N = 3) and huDRAG-A2 (N = 3) mice at 4 weeks post-infection with H37Rv Mtb. One huNRG mouse was removed from lung CFU counts due technical error.

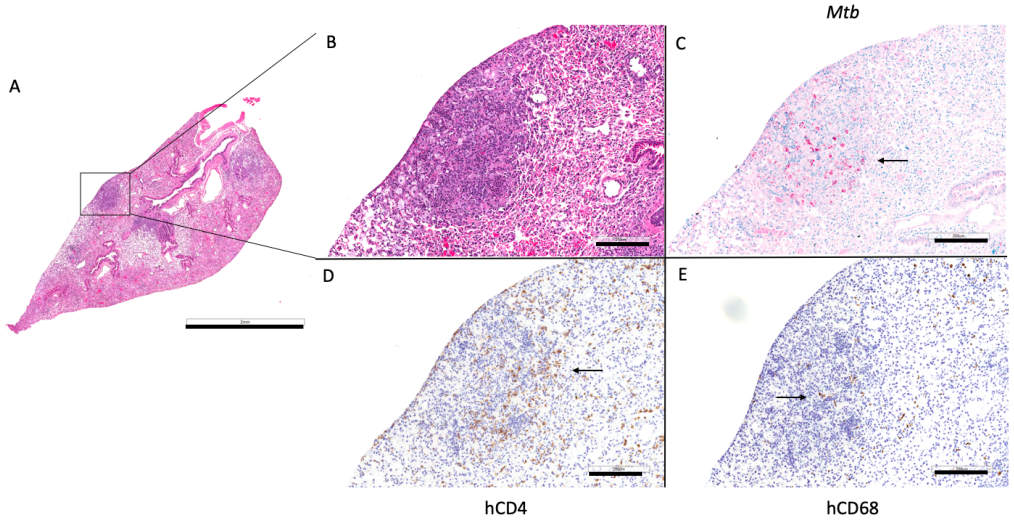


Figure 10. Mtb-infected huNRG **(A)** Whole lung section H&E (2x). **(B)** H&E of granuloma (10x). **(C)** Acid fast bacilli staining of granuloma (10x), arrow indicate Mtb bacilli stained red, **(D)** human CD4+ IHC (10x), arrow indicates human CD4+ T cells stained brown, **(E)** human CD68+ IHC (10x), arrow indicates human CD68+ macrophages stained brown. (black scale bars for 2x = 1mm; 10x = 200μm).

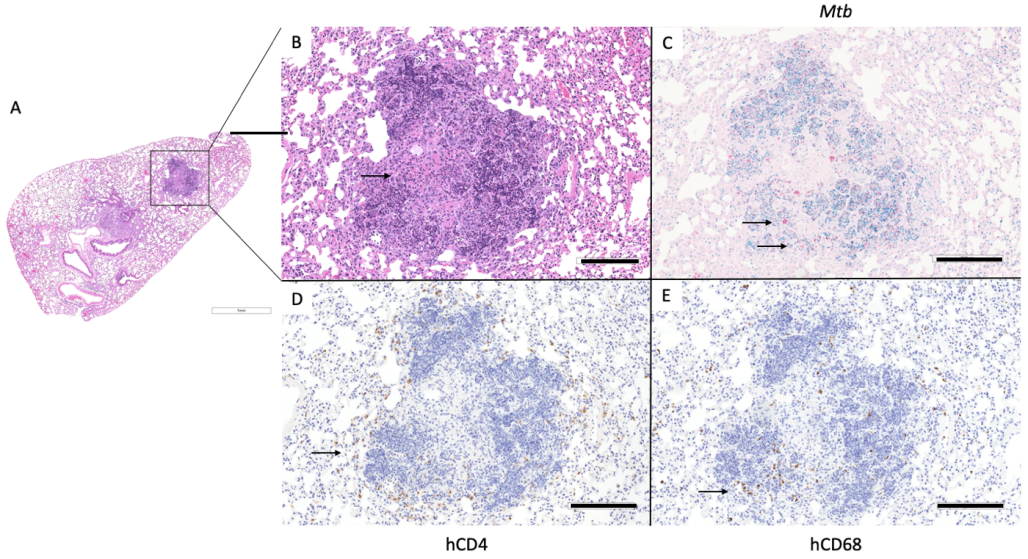


Figure 11. Mtb-infected huDRAG-A2 **(A)** Whole lung section H&E (2x). **(B)** H&E of granuloma (10x). **(C)** Acid fast bacilli staining of granuloma (10x), arrow indicate Mtb bacilli stained red, **(D)** human CD4+ IHC (10x), arrow indicates human CD4+ T cells stained brown, **(E)** human CD68+ IHC (10x), arrow indicates human CD68+ macrophages stained brown. (black scale bars for 2x = 1mm; 10x = 200μm).

Conclusions & Significance

HIV:

- HuDRAG-A2 mice reconstitute with significantly higher counts of B cells and HIV target cells in the blood including human CD4+ T cells and CD14 monocytes/macrophages compared to huNRG.
- Both huNRG and huDRAG-A2 mice sustains HIV infection in plasma but huDRAG-A2 mice may show more severe CD4+ T cell depletion than huNRG.
- 8 weeks post-HIV infection, hCD4+ T cells in the vaginal mucosa and lung tissue are depleted in both models.

TB:

- Both models are successfully infected in the lung with Mtb with bacteria dissemination into spleen tissue.
- HuDRAG-A2 lungs develop human-like lung histopathology where organized granulomas contain a caseating necrotic core that is surrounded by a halo of CD4+ T cells.

Significance & Future direction:

- Given the high morbidity associated with HIV/TB co-infection, more research is necessary to understand the mechanisms of immune response and pathogenesis in vivo.
- HuNRG and huDRAG-A2 showed the ability to well-model HIV and TB infection alone, while the hu-DRAGA2 model demonstrated greater potential overall in recapitulating human immune responses.
- On-going studies in our lab are using both models in HIV/TB co-infection, and future studies will explore HIV ART treatment and TB vaccination within the models.