Comparative Investigation of Immunopathogenesis in HIV- and *Mycobacterium Tuberculosis* Single Infection Versus Co-Infection Using Humanized Mice

Miaoyun Zhao, Subhur Mandal, Yilun Cheng, Qingsheng Li

The Wistar Institute

Co-infection of HIV-1 with *Mycobacterium tuberculosis* (*Mtb*) remains a major global health challenge, yet the cellular and tissue-level features of dual infection in a human immune context remain incompletely defined. Here, we used a human immune system (HIS) mouse model based on NSG mice engrafted with human $CD34^+$ hematopoietic stem progenitor cells to investigate pulmonary and extrapulmonary pathogenesis in HIV-1 or *Mtb single infection* versus *their* co-infection. Four weeks after infection, *Mtb* burdens detected with Kinyoun staining and *Mtb antibody* and HIV loads using RNAscope exhibited higher than single infections, demonstrating reciprocal enhancement of replications. Further, more collagen deposition and structural damage was observed in co-infected lungs compared with HIV-1– or *Mtb*-only groups. RNA-sequencing of lung tissues demonstrated that co-infection has a distinct transcriptional profile enriched for extracellular-matrix organization, keratinization, calcium signaling, and cell-adhesion pathways. Multiplexed spatial imaging (CODEX combined with RNAscope further revealed enlarged inflammatory lesions in co-infected lungs, with increased accumulation and altered spatial organization of $CD68^+$ macrophages relative to *Mtb*-only infection. HIV-1 vRNA–positive signals were frequently detected in macrophage-enriched regions. Finally, HIV-1 vRNA and *Mtb* were also detected in extrapulmonary organs, i.e. kidney and spleen, however only vRNA was detected but not *Mtb* in brain tissues, indicating that *Mtb* systemic infection in this model. Together, these findings show that HIV and *Mtb* co-infection reciprocally increased pathogenesis of both pathogens, providing a tractable platform to investigate human immune responses during dual infection.