Mechanisms regulating granuloma development and heterogeneity in high- and low-transmission *Mycobacterium tuberculosis* infections

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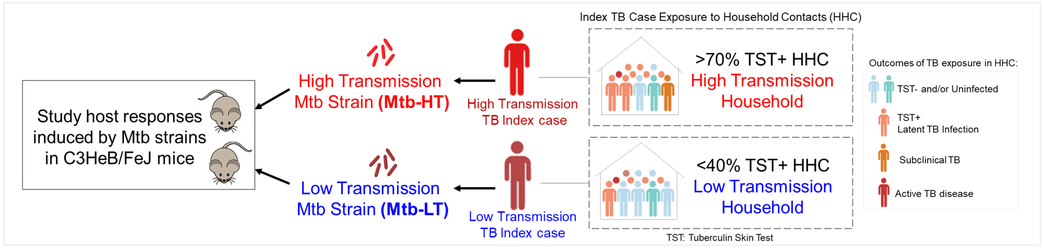
RATIONALE

- The tremendous global burden of Tuberculosis (TB) is sustained due to the continued cycle of *Mycobacterium tuberculosis* (Mtb) transmission and infection.
- A prominent histopathological feature of TB is the formation of granulomas, driven by both bacterial and host factors in the lungs of infected hosts.
- While granulomas serve to contain Mtb in the lung, they can undergo necrosis and can cavitate into the airways, facilitating bacterial dissemination and transmission.
- Distinct early host immune mechanisms induced by various Mtb strains are associated with variability in subsequent Mtb transmission.
- Defining these mechanisms may offer substantial opportunities for therapeutic interventions to mitigate Mtb transmission.

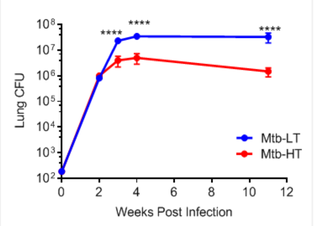
STUDY DESIGN

Study of Household Contacts (HHC) of Index TB cases (N=124) conducted in Vitória, Brazil.



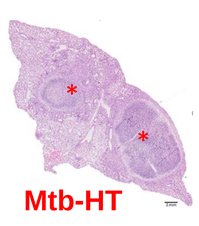
BACKGROUND

- We previously reported that in Mtb-HT-infected mice, rapid IL-1receptor-dependent alveolar macrophage migration from the alveolar space into the interstitium led to early dissemination of bacteria to the lymph nodes, Th1 priming and formation of caseating necrotic granulomas.
- In contrast, this dissemination was delayed in Mtb-LT infection, promoting the development of a Th17 response, diffused inflammatory lung pathology, and significantly increased bacterial burden.



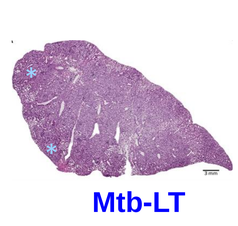
- Bacterial burden is significantly increased in Mtb-LT-infected mice after Week 2 post infection

CFU: Colony Forming Units



Mtb-HT

- *Caseating necrotic lung lesions
- Increased bacterial dissemination to lymph nodes
- T-Helper cell 1 (Th1) priming



Mtb-LT

- *Diffused infiltrative lung pathology
- Delayed bacterial dissemination to lymph nodes
- T-Helper cell 17 (Th17) response

Arianne Lovey et. al, Early alveolar macrophage response and IL-1R-dependent T cell priming determine transmissibility of Mtb strains. Nature Communications, Feb 2022

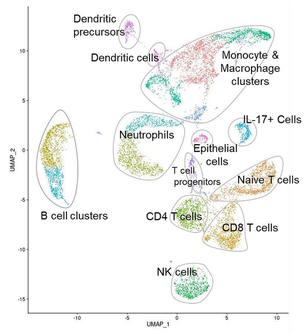
STUDY AIM

We hypothesize that the distinct immunopathology of Mtb-HT- and Mtb-LT-infected lungs underlie variability in transmission potential. We aim to characterize the distinct early host immune mechanisms induced by Mtb-HT and Mtb-LT strains in the C3HeB/FeJ mice that develop caseating necrotic lesions similar to human TB.

RESULTS

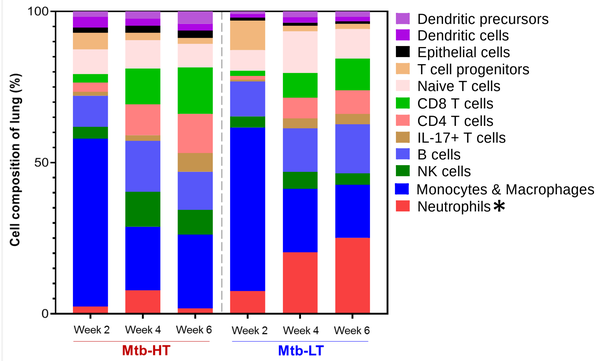
To examine the evolving lung immune cell landscape that shapes the distinct immune responses in Mtb-HT and Mtb-LT infections, we conducted single cell RNA-sequencing (10x Genomics) of lung cell infiltrates at 2-, 4- and 6-weeks post Mtb-HT and Mtb-LT infections.

1. Identification of cell clusters using single cell RNA-seq



UMAP plot: Uniform Manifold Approximation and Projection

2. Comparing cell clusters at weeks 2, 4 and 6 post Mtb infection



3. Neutrophils are increased in Mtb-LT-infected mice at Week 6 post Mtb infection

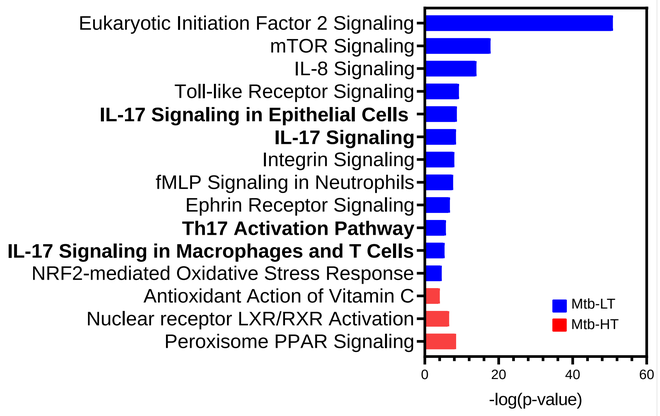
✻ Neutrophils (% of total lung cells)

Mtb-HT			Mtb-LT		
Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
2.36	7.69	**1.81**	7.53	20.3	**25.13**

1841 Differentially expressed genes between Mtb-HT and Mtb-LT Neutrophils at Week 6

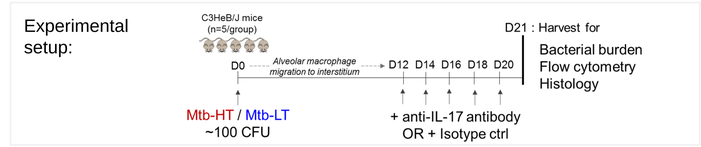
Identify top differentially expressed canonical pathways

4. Ingenuity Pathway Analysis: Several IL-17 pathways upregulated by Mtb-LT-Neutrophils at Week 6

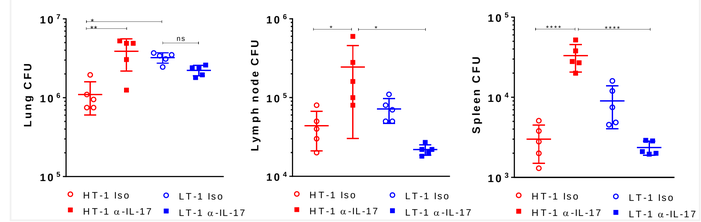


5. Ongoing studies: Early impact of blocking IL-17

If IL-17 signaling is inhibited before Week 2, we hypothesize that Mtb-LT-infected will mice exhibit reduced bacterial burden similar to Mtb-HT mice.



Preliminary data (Bacterial burden at D21):



CFU: Colony Forming Units | Iso: Isotype antibody control | α-IL-17: anti-IL-17 blocking antibody
Significance determined using one-way ANOVA with Tukey's correction, alpha set at p<0.05

CONCLUSIONS

- In comparison to Mtb-HT-infected mice, Mtb-LT-infected mice demonstrated increased number of neutrophils at Week 6 post infection. These neutrophils upregulated pathways involved in IL-17 signaling and pathways promoting bacterial replication.
- By inhibiting IL-17 signaling early-on post Mtb infection, we observed a reduction in bacterial burden in the Mtb-LT-infected mice. Surprisingly, the bacterial burden was significantly increased in Mtb-HT-infected mice upon IL-17 blocking, suggesting that while the amount of IL-17 involved may differ, **IL-17 is a key regulator of bacterial replication** in both infections.
- Our on-going studies are focused on characterizing kinetics and the early impact of IL-17 signaling on bacterial burden, neutrophil recruitment and differences in granuloma development in Mtb-HT and Mtb-LT infections.
- Our study will provide key insights into human TB disease manifestations induced by heterogenous Mtb strains.

ACKNOWLEDGEMENTS

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