A *Mycobacterium tuberculosis* variable antigen vaccine induces infection tolerance

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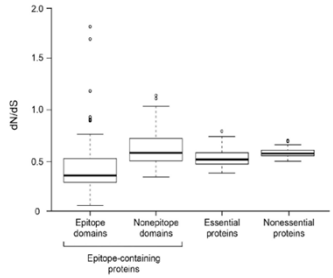
1 T cell epitope conservation in Mtb

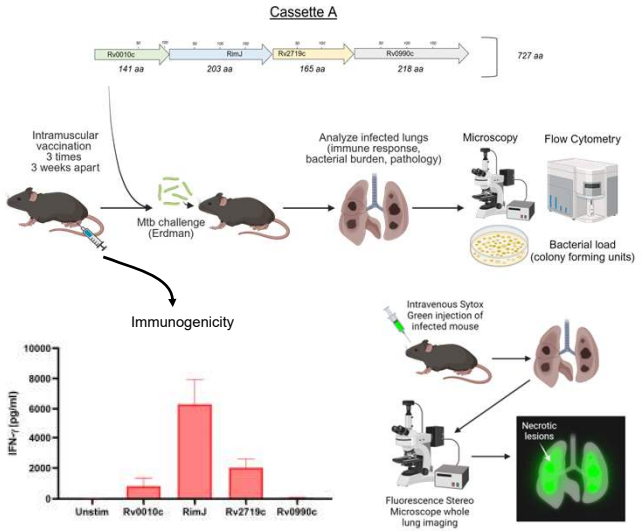
A majority of *Mycobacterium tuberculosis* (Mtb) T cell antigens are hyperconserved which may suggest insufficient selection pressure from T cell responses during infection[1]. Antigens exhibiting sequence diversity within T cell epitopes have been identified through an analysis of genomes from 200+ Mtb strains.



Genetic diversity of human Mtb T cell epitopes. The substitution rates (dN/dS) in epitopes and other elements of the *Mycobacterium tuberculosis* complex (MTBC) genome from 216 Mtb strains representative of seven phylogenetic lineages (from Coscolla M, et al. 2015. *Cell Host Microbe*).

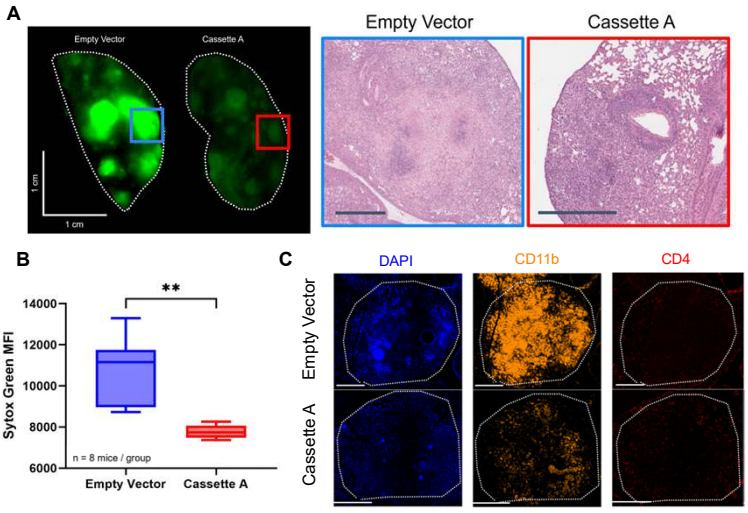
Hypothesis: Antigens exhibiting sequence variation due to immunological pressure induce unique T cell responses that promote enhanced control of Mtb infection.

2 Investigating the impact of variable antigen T cell responses using a DNA vaccine



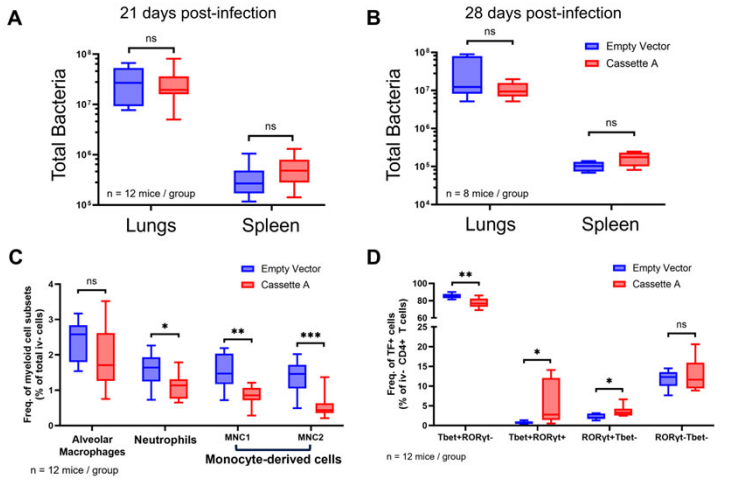
A DNA vaccine encoding for the sequence variable antigens (Rv0010c, RimJ, Rv2719c, and Rv0990c) was generated with the pVAX1 vector and used to vaccinate susceptible SP140$^{-/-}$ mice[2] to determine the impact of T cell responses to the variable antigens during Mtb infection. High parameter flow cytometry was used to assess the immune cell composition at 21 days post-infection. Pathology was assessed by histology, immunofluorescence, or whole lung Sytox Green fluorescence at 28 days post-infection[3].

3 Variable antigen vaccination reduces lung necrosis



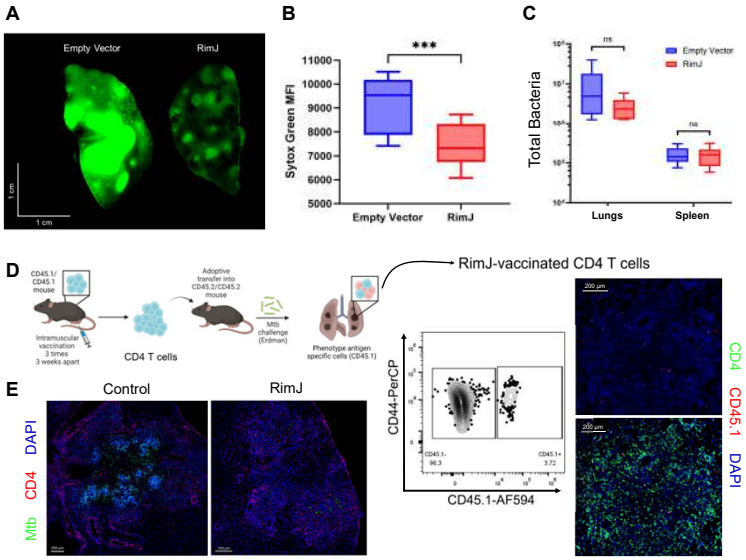
Cassette A vaccination of SP140$^{-/-}$ mice reduces necrosis. SP140$^{-/-}$ mice vaccinated with empty vector or Cassette A were challenged with 50-100 CFU of Mtb Erdman. A) Representative images of infected lungs from Sytox Green injected mice[3] and corresponding H&E sections (scale bar = 500 μm). B) The MFI of each side of the lungs was quantified using ImageJ. C) Representative immunofluorescence images of granulomas from lungs at 28 days post-infection (scale bar = 250 μm). Data was compared using a t-test and * denotes p-values < 0.05.

4 Variable antigen vaccination alters the immune response to infection in SP140$^{-/-}$ mice



Cassette A vaccination of SP140$^{-/-}$ mice reduces disease burden without affecting bacterial loads. SP140$^{-/-}$ mice vaccinated with empty vector or Cassette A were challenged with 50-100 CFU of Mtb Erdman. A and B) Bacterial burdens in the lungs and spleens at day 21 and 28 post-infection. C and D) Flow cytometry data is the mean ± SEM frequency of cell types from infected lungs at 21 days post-infection. Data was compared using a t-test and * denotes p-values < 0.05.

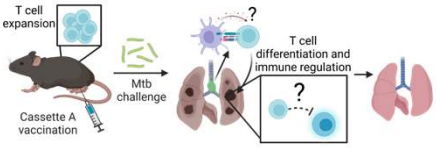
5 Characterizing the CD4 T cell response to RimJ



RimJ as a single antigen to investigate the CD4 T cell response to variable antigens. A-C) SP140-/- mice vaccinated with either the empty vector or RimJ were challenged with 50-100 CFU of Mtb Erdman. The lungs were processed at 28 days post-infection, as described for Cassette A vaccination experiments, to assess A-B) necrosis and C) bacterial burdens (10 mice per group). D) Experimental diagram for adoptive transfer of CD4 T cells from RimJ-vaccinated mice with flow cytometry and representative immunofluorescence showing CD45.1+ RimJ-specific CD4 T cells at 28 days post-infection. E) Representative immunofluorescence of a control lesion with necrosis (left) compared to a non-necrotic lesion from a mouse that received RimJ CD4 T cells (right). Data was compared using a t-test and * denotes p-values < 0.05.

6 Conclusions and Future Directions

- Cassette A or RimJ vaccination decreases pathology without reducing bacterial burden and induces significant changes in the T cell and myeloid cell compartments.
- Phenotyping and tracking of RimJ-specific T cells is possible with a congenic adoptive transfer.
- Future experiments aim to characterize the function of RimJ-specific T cells during Mtb infection.



Acknowledgements
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Ernst Lab

References:
1. Coscolla M, et al. 2015. Cell Host Microbe.
2. Ji DX and Witt KC, et al. 2021. eLife.
3. Amaral EP, et al. 2019. J Exp Med.
4. Bankhead, P. Et al. 2017. Sci Rep.