T cell responses to Mycobacterium tuberculosis variable antigens

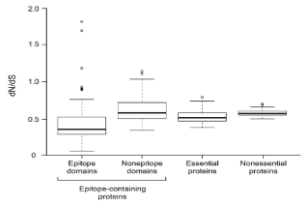
Zachary P. Howard, Paul Ogongo, I-Chang Chang, Mary Beth Moreno, Weihao Zheng, and Joel D. Ernst

Division of Experimental Medicine, Department of Medicine, University of California San Francisco, 1001 Potrero Avenue, CA 94110

UCSF Experimental Medicine
Department of Medicine

T cell epitope conservation in Mtb

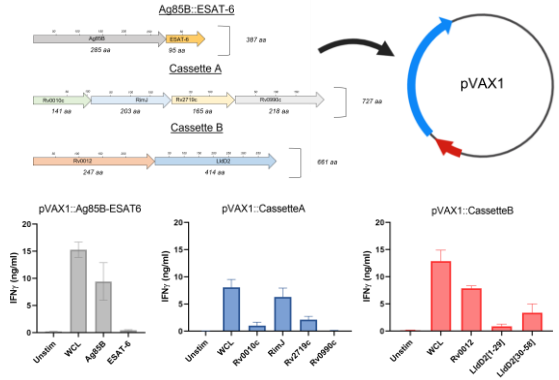
- A majority of *Mycobacterium tuberculosis* (Mtb) T cell antigens are hyperconserved which may suggest insufficient selection pressure from T cell responses during infection[1]
- An analysis of genomes from 200+ Mtb strains from multiple lineages identified 6 candidate Mtb sequence variable T cell antigens that may induce more protective T cell responses during infection



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 using genomes from 216 Mtb strains representative of the seven known human-adapted phylogenetic lineages of the MTBC (from Coscolla M, et al. 2015. *Cell Host Microbe*).

Hypothesis: Antigens exhibiting sequence variation due to immunological pressure induce T cell responses that are more protective than T cell responses against sequence conserved antigens and provide enhanced control of Mtb infection.

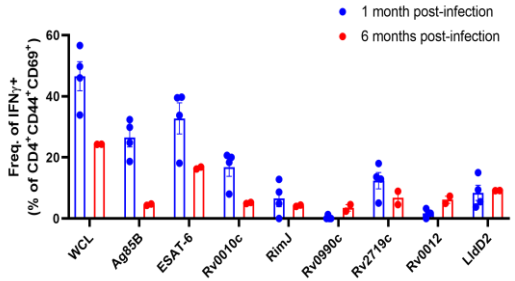
Designing a DNA vaccine using variable antigens



A DNA vaccine encoding for the candidate sequence variable antigens is immunogenic in C57BL/6 mice. C57BL/6 mice (n = 5 per vaccine) were vaccinated (i.m.) 3 times, 3 weeks apart. 3 weeks after the final vaccination, spleens were collected from each mouse and splenocytes were stimulated *ex vivo* for 72 hours with 10 μg/ml of pooled overlapping peptides (18mers overlapping by 11 amino acids) for each of the vaccine antigens, 10 μg/ml Mtb whole cell lysate (WCL), or left unstimulated (unstim). IFNγ concentrations in the supernatant was measured by ELISA.

Variable antigens induce lung CD4+ T cell responses

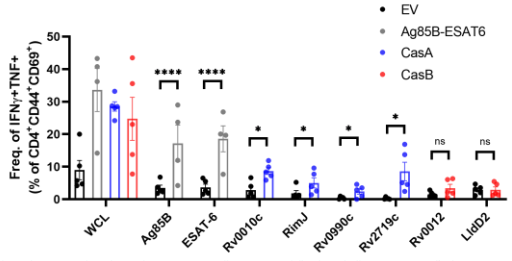
Variable antigen-specific CD4+ T cell responses are detectable in the lungs during Mtb infection at 1- and 6-months post-infection.



Variable antigens induce lung CD4+ T cell responses during Mtb. Lung cells from Mtb H37Rv-infected C57BL/6 mice at 1 month (blue, n = 4) and 6 months (red, n = 2) post-infection were stimulated *ex vivo* with pooled overlapping peptides for 12 hours in the presence of Monensin/Brefeldin A. Intracellular cytokine staining and flow cytometry was used to determine the frequency of IFNγ+ CD4+ T cells.

Variable antigen DNA vaccines boost the CD4+ T cell response after challenge

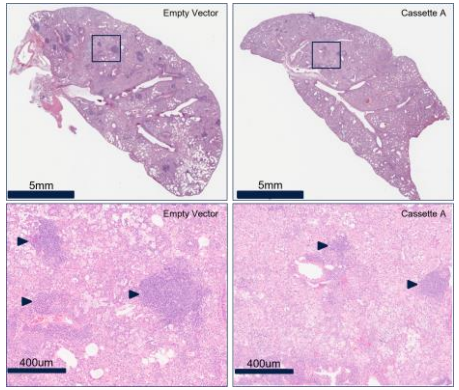
Variable antigen-specific lung CD4+ T cell responses are boosted in vaccinated mice following challenge with Mtb Erdman.



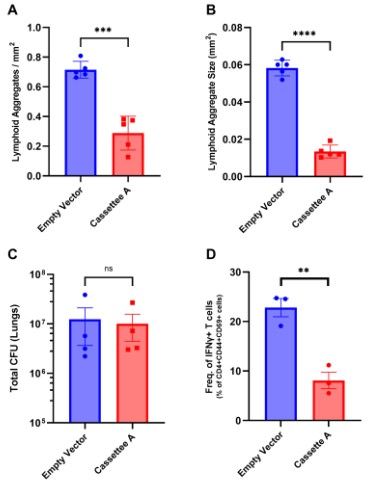
Variable antigens vaccines boost lung CD4+ T cell responses following challenge. Lung cells from empty vector- (EV), Ag85B-ESAT6-, Cassette A- (CasA), and Cassette B- vaccinated (CasB) and Mtb Erdman-infected C57BL/6 mice 1-month post-challenge were stimulated *ex vivo* with the designated pooled overlapping peptides for 12 hours in the presence of Monensin/Brefeldin A. Intracellular cytokine staining and flow cytometry was used to determine the frequency of IFNγ+ CD4+ T cells. Data is mean ± SEM and a 2way ANOVA with a Tukey's multiple comparison test was performed in GraphPad Prism (**** = p-value<0.0001, * = p-value<0.05).

Variable antigen DNA vaccines alter the immune response to infection

Mice vaccinated with Cassette A displayed reduced IFNγ responses to Mtb whole cell lysate (WCL) and a reduced frequency and size of lymphoid aggregates in the lung as compared to mock-vaccinated mice.



Cassette A vaccination alters immunopathology in the lungs after challenge with Mtb. Lungs from male C57BL/6 mice vaccinated with either the empty vector or Cassette A were analyzed by H&E staining 3 months post-challenge with Mtb Erdman. Black squares denote inset shown below each larger image and black triangles denote lymphoid aggregates.

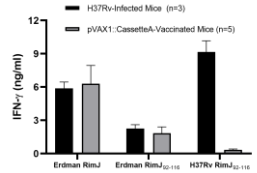


Cassette A vaccination reduces lymphoid aggregates but not bacterial burden after challenge with Mtb Erdman. Lungs from Empty Vector- or Cassette A-vaccinated C57BL/6 mice were analyzed by H&E staining 3 months post-challenge with Mtb Erdman. A and B) Cassette A-vaccinated mice had a reduced frequency and size of lymphoid aggregates as compared to the Empty Vector-vaccinated mice, but C) no difference in bacterial burden in the lungs. D) Cassette A-vaccinated mice had a reduced frequency of lung IFNγ+ CD4+ T cells responding to ex vivo stimulation with Mtb whole cell lysate (background response subtracted). Data is mean ± SEM and a Welch's t test was performed for histology (n=5 sections), CFUs (n=4 mice), and T cell responses (n=4 mice) (** = p-value<0.01, *** = p-value<0.001, **** = p-value<0.0001, ns = not significant).

Naturally-occurring epitope variation impacts the T cell response to RimJ

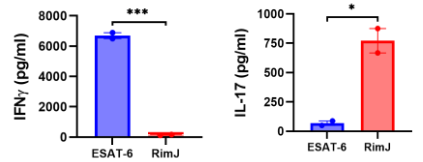
A substitution in a predicted T cell epitope (highlighted) in Mtb Erdman impacts the magnitude of the T cell response during *ex vivo* stimulation of splenocytes from Mtb-infected and vaccinated mice.

Antigen	Start	End	Peptide	Adj. Percentile
RimJ H37Rv	100	114	SAWIGYWVPSAATGG	0.90
RimJ Erdman	100	114	SAWIGCWVPSAATGG	3.95



A single substitution in a RimJ epitope effects T cell responses. MHC-II binding predictions were performed for the I-A(b) allele through IEDB[2,3]. Splenocytes from Mtb H37Rv-infected or Cassette A-vaccinated C57BL/6 mice were stimulated *ex vivo* with RimJ pooled overlapping peptides matching the Mtb Erdman sequence (Erdman RimJ) or two peptides matching the H37Rv or Erdman sequence spanning the dominant T cell epitope (amino acids 92-116) for 72 hours and IFNγ concentrations in the supernatant were measured by ELISA.

Differential cytokine responses to RimJ occur in the lung draining lymph nodes during infection in mice



RimJ induces an IL-17 response in the mediastinal lymph node during Mtb infection. Mediastinal lymph nodes were pooled from 5 female Mtb H37Rv-infected CB6F1/J mice 1-month post-infection and cells were stimulated *ex vivo* with overlapping peptides for ESAT-6 (blue) or RimJ (red) in duplicate for 72 hours. IFNγ and IL-17 concentrations in the supernatant by ELISA. Data is mean ± SD and a one-way ANOVA with a Tukey's multiple comparison test was performed in GraphPad Prism (*** = p-value<0.001, * = p-value< 0.05). Results are representative of two independent experiments.

Conclusions/Future Directions

- Variable antigens induce detectable CD4+ T cell responses during infection that can be boosted with a DNA vaccine
- A DNA vaccine encoding for variable antigens alters immunopathology and T cell responses to infection
- RimJ induces a differential, IL-17 dominated, response in the mediastinal lymph nodes during infection
- Further investigate the RimJ IL-17 response during infection and after vaccination with a Th17-polarizing vaccine

Acknowledgements

Ernst Lab
UCSF Biomedical Sciences PhD Program
My thesis committee: Babak Javid, Jason Cyster, Adrian Erlebacher

References:

1. Coscolla M, et al. 2015. Cell Host Microbe.
2. Wang P, et al. 2008. PLoS Comput Biol.
3. Wang P, et al. 2010. BMC Bioinformatics.

Conflicts of interest: None