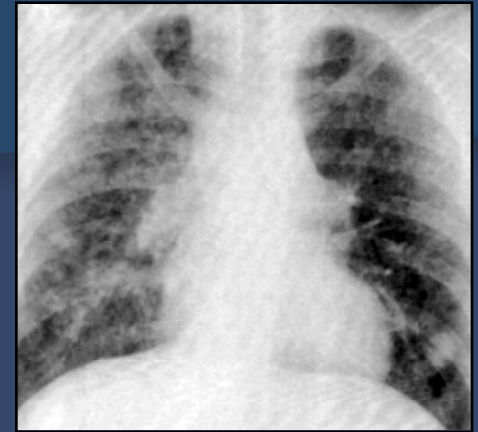


The Second Global Forum on TB Vaccines



Recognition of Mtb Infected Cells:

Implications for Vaccine Design

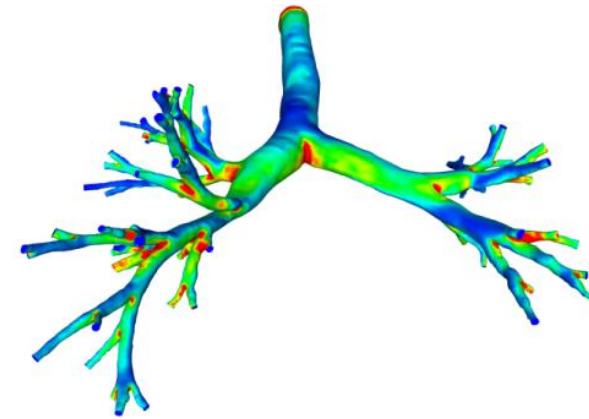
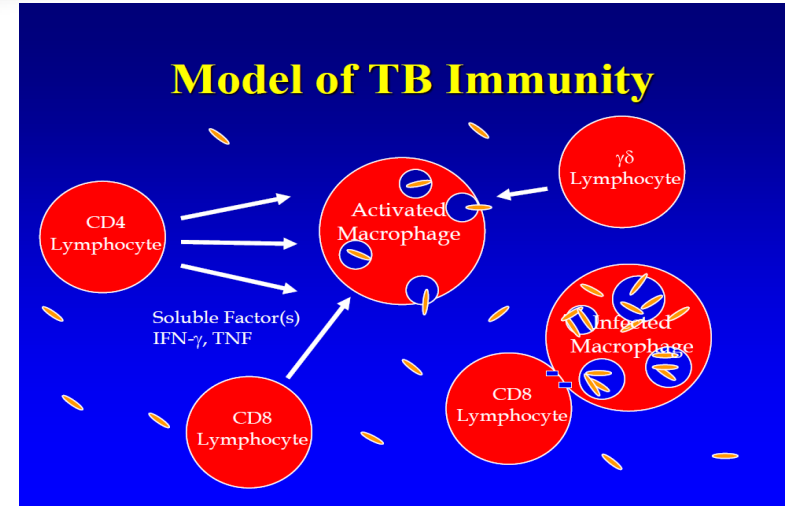
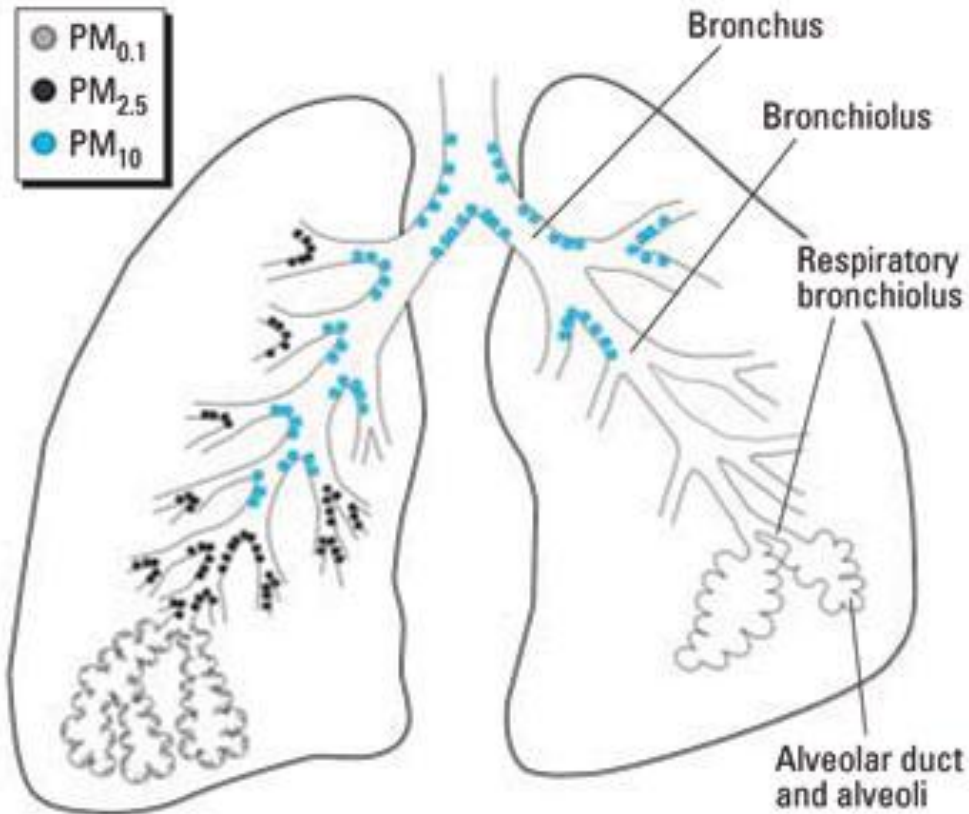
David Lewinsohn, MD, PhD
Pulmonary & Critical Care Medicine

Portland VA Medical Center
Oregon Health & Sciences University

In Humans, Where does Mtb Reside?

- Molecular evidence of dormancy
 - Cornell model
 - Lillebaek T et. Al., Stability of DNA patterns and evidence of Mycobacterium tuberculosis reactivation occurring decades after the initial infection. J Infect Dis 2003; 188: 1032–1039.
 - Mtb can be recovered in 12-45% of tissue from those without apparent disease
 - Not necessarily in granulomas
 - TB has resulted from organ transplantation
 - Clustering less likely in immigrants
 - However, risk is highest in the first two years following immigration

Distribution of Particulates in the Lung

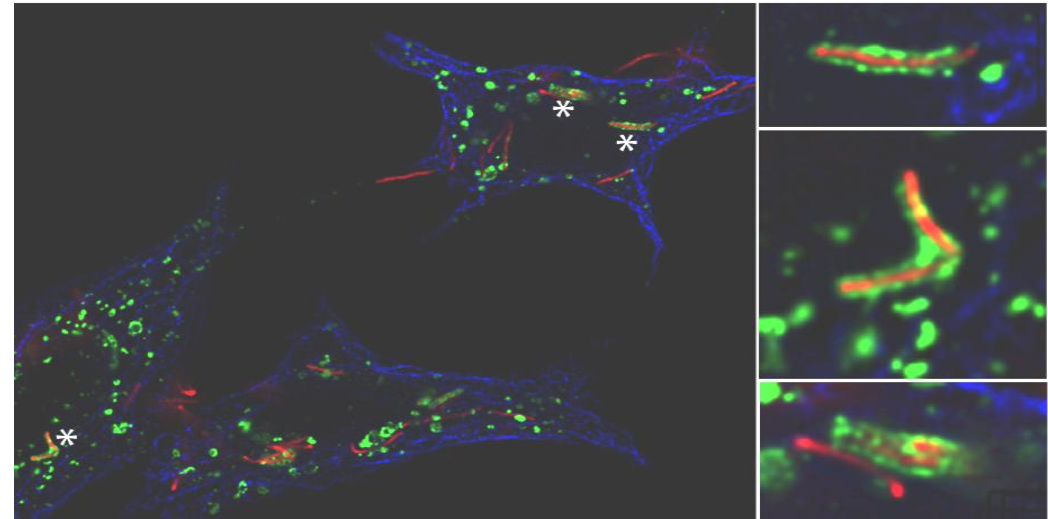


Mtb and the Lung Epithelium

- Bermudez, & Goodman, Infect Immun 1996. Mycobacterium tuberculosis Invades and Replicates within Type II Alveolar Cells
- Hernandez-Pando R, et al., Lancet 2000. Persistence of DNA from Mycobacterium tuberculosis in Superficially Normal Lung Tissue During Latent Infection. Lancet 356: 2133-2138.
- Menozzi et al., JEM 1996. Identification of a Heparin-binding Hemagglutinin Present in Mycobacteria
 - HBHA mediates adherence to epithelial cells
- Hannah et al., Science 2010. Tuberculous Granuloma Induction via Interaction of a Bacterial Secreted Protein with Host Epithelium
 - Esat6 dependent release of MMP9 by adjacent epithelial cells
- Desvignes et al., Immunity 2009, Interferon- γ -Responsive Nonhematopoietic Cells Regulate the Immune Response
- to Mycobacterium tuberculosis
 - Absence of IFN- γ R1 on non-hematopoietic cells resulted in:
 - Increased neutrophilic inflammation
 - Decreased indoleamine-2,3-dioxygenase
 - Increased IL-17
- Olubisi et al., 2010. Journal of Medical Microbiology. Adhesion to and invasion of pulmonary epithelial cells by the F15/LAM4/KZN and Beijing strains of Mycobacterium tuberculosis

Gold et al., 2010 PLoS Biology , Human Mucosal Associated Invariant T Cells Detect Bacterially Infected Cells

- Non-classically restricted T cells predominate in those unexposed to Mtb - MAIT
- Recognize Mtb-infected myeloid and non-myeloid cells
- Enriched in the lung and airway
- Absent from the blood of those with TB
- Mtb-infected epithelial cells are recognized by CD8+ T cells (both classically and non-classically restricted)

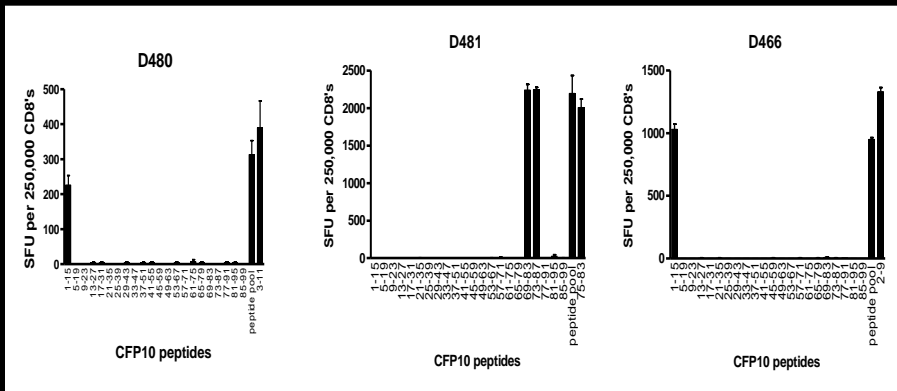


Lessons from HLA-Ia Restricted CD8 T Cell Clones & Large Scale Identification of CD8 Antigens

2005-2009 NIH HHSN272200900053C

2009-2014 HHSN266200400081C

- HLA-B restriction is common
- Epitopes are often 10 or 11aa in length
- Immuno-dominance
 - Both within an Antigen
 - And – Across the Genome
 - At best, good antigens are seen in about 50%
- Novel antigens
 - EsxJ Family PPE15
 - PE9 PPE51
 - PE/PGRS 42 ctpF
- New HLA prediction tools accurate
- Non-classical cells predominate in those without Mtb infection
 - HLA-E
 - MRI



Clone ^a	Gene	Accession Number	HLA-Restricting Allele	Epitope Location	Epitope Sequence	#SFU/250,000 CD8+ T-cells	MHC Binding Affinity (IC50 nm)	Vbeta region	Tetramer available?
D 160 1-1B ^b (0)	CFP10	Rv3874	B44	2-11	AEMKTDAATL	360	38	IND	no
D160 1-6F ^b (0)	CFP10	Rv3874	B14	85-94	RADEEQQQAL	120	NA	TBD	no
D432 H12 (1)	CFP10	Rv3874	B3514	49-58	TAAQAAVVRF	258	2011 ^c	5.3	yes
D466 A10 (9)	CFP10	Rv3874	B4501	2-9	AEMKTDA	2458	48	IND	yes
D466 D6 (0)	CFP10	Rv3874	B4501	2-12	AEMKTDAATLA	1993	6.2	22	yes
D481 C10 (8)	CFP10	Rv3874	B1502	75-83	NIRQAGVQY	1715	14 ^d	9	yes
D481 C11 (0)	CFP10	Rv3874	B1502	75-83	NIRQAGVQY	1715	14 ^d	13.6	yes
D480 F6 (5)	CFP10	Rv3874	B0801	3-11	EMKTDAATL	387	79	13.1	yes
D571 B12 (2)	CFP10	Rv3874	B4402	2-11	AEMKTDAATL	31	38	IND	no
D571 E9 (3)	CFP10	Rv3874	B4402	2-11	AEMKTDAATL	31	38	14	no
D504 E4 (0)	Mtb9.8	Rv0287	A0201	3-11	LLDAHIPQL	72	0.39	8	yes
D454 B10 (0)	Mtb9.8	Rv0287	B0801	53-61	AAHARFVAA	88	0.22	IND	yes
D454 H1-2 (0)	Mtb8.4	Rv1174 c	B1501	33-43	AVINTTCNYGQ	24	10	7.1	yes
D432 A3 (1)	Mtb 8.4	Rv1174 c	B3514	61-69	ASPVAQSYL	210	127 ^c	14	yes
D443 H9 (0)	Ag85B	Rv1886 c	B4102	144-153	ELPQWLSANR	<10	N/A	22	no
D504 F9 (5)	EsxJ*	Rv1038 c	B5701	24-34	QTVEDEARRMW	84	10	Indeterminate	yes
D432 D8 (0)	PE9	Rv1088	B3905	53-67	RLFNANAEYHALSA	94	TBD	8	no
D432 H8 (0)	PE_PGR S42	Rv2487 c	B3514	48-56	SAAIAGLFG	78	TBD	7.1	yes

Lewinsohn et al., PLoS Pathogens, 2007

A Phase I, Double-Blind, Randomized, Placebo-controlled Leukapheresis Study to Obtain Lymphocytes for the Study of Immune Responses in Healthy Adult Volunteers in the U.S. who receive BCG Vaccination followed by Boosting with AERAS-402

Investigational Product: AERAS-402

Aeras Protocol Number: C-021-402

US FDA IND Number: BB-IND 13140

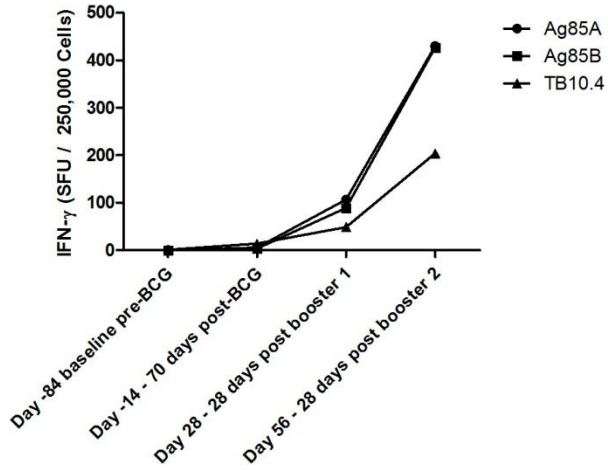
- Oct 2009 - Present
 - 11 Healthy Subjects
 - QFT - at baseline
 - Up to 3 Placebo Controls

<u>Evaluation</u>	<u>Screen</u>	<u>Day -84</u>	<u>Day -14</u>	<u>Day 0</u>	<u>Day 28</u>	<u>Day 56</u>	<u>Day 98</u>
QuantiFERON	x						
BCG administration		x					
Aeras-402/ placebo				x	x		
Aeras-402 neutralization				x			
ICS/Elispot samples		x	x		x	x	
Leukapheresis			x				x

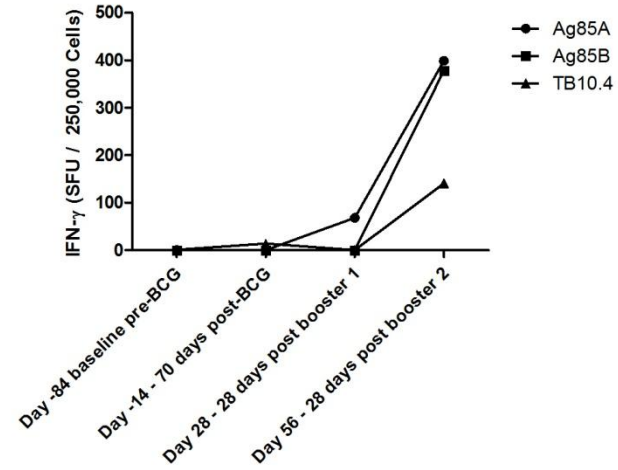


Ex-Vivo Responses to Aeras-402 Vaccine

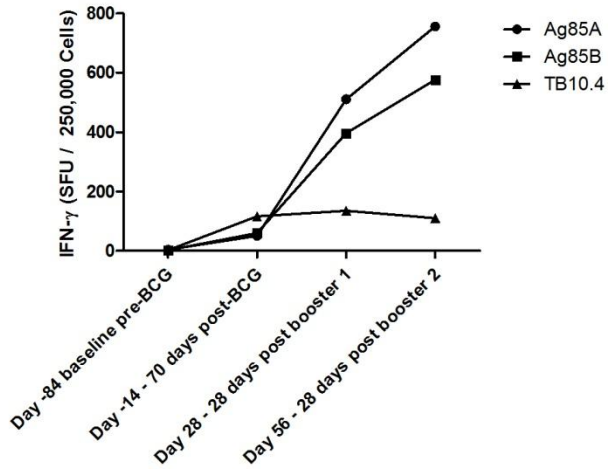
Subject 2 PBMC



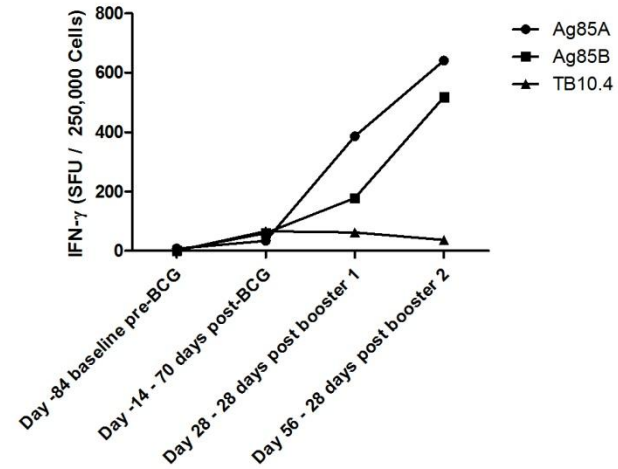
Subject 2 CD8



Subject 8 PBMC

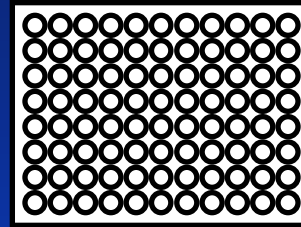
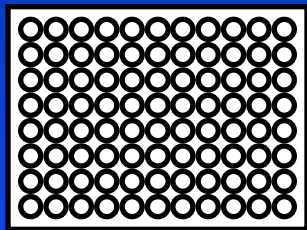


Subject 8 CD8

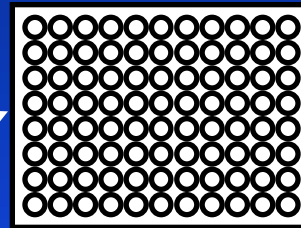


Limiting Dilution Analysis

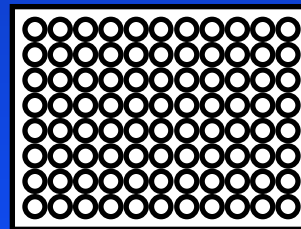
Purified CD8⁺ T Cells*
Irradiated PBMC
Peptide Pulsed DC
IL-2 (0.5 ng/ml)



Autologous DC
No Mtb



Autologous DC
+ Peptide Pool



Autologous DC
+ Mtb

Expansion/ Characterization
of Selected Clones

* CD8⁺ T cell number estimated by precursor frequency analysis

Limiting Dilution Cloning – Subject 2

- Initial screen results:
 - 5 clones responded to Ag85A
 - 5 clones responded to Ag85B
 - 4 clones responded to Tb10.4
 - None responded to Mtb
 - 14 clones expanded and rescreened
 - 6 retain antigen reactivity

Evaluation of Clones

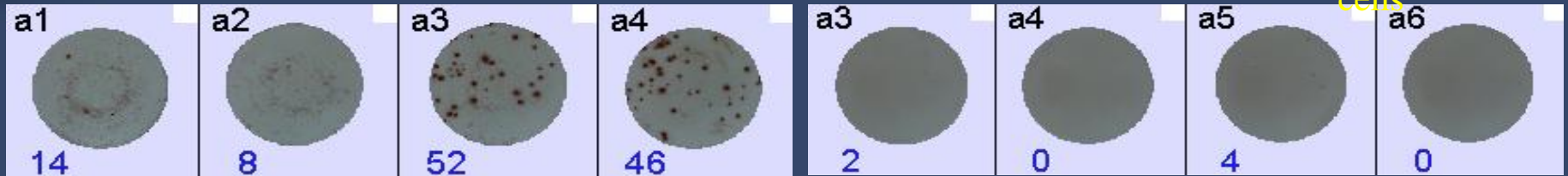
Media

Peptide

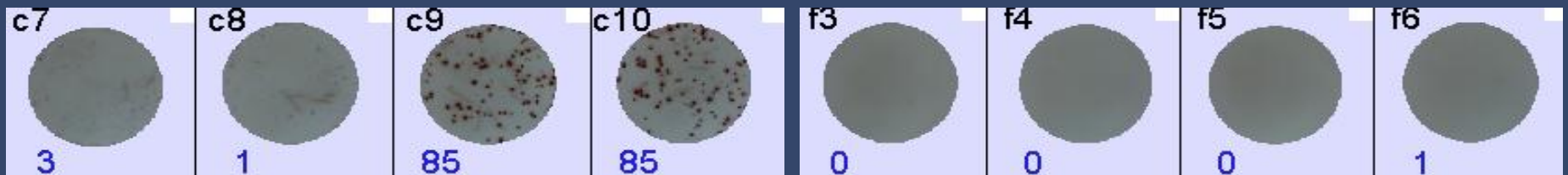
Mtb + 5,000 T-cells

Mtb + 20,000 T-cells

D002
E1-1



D002
E3-3

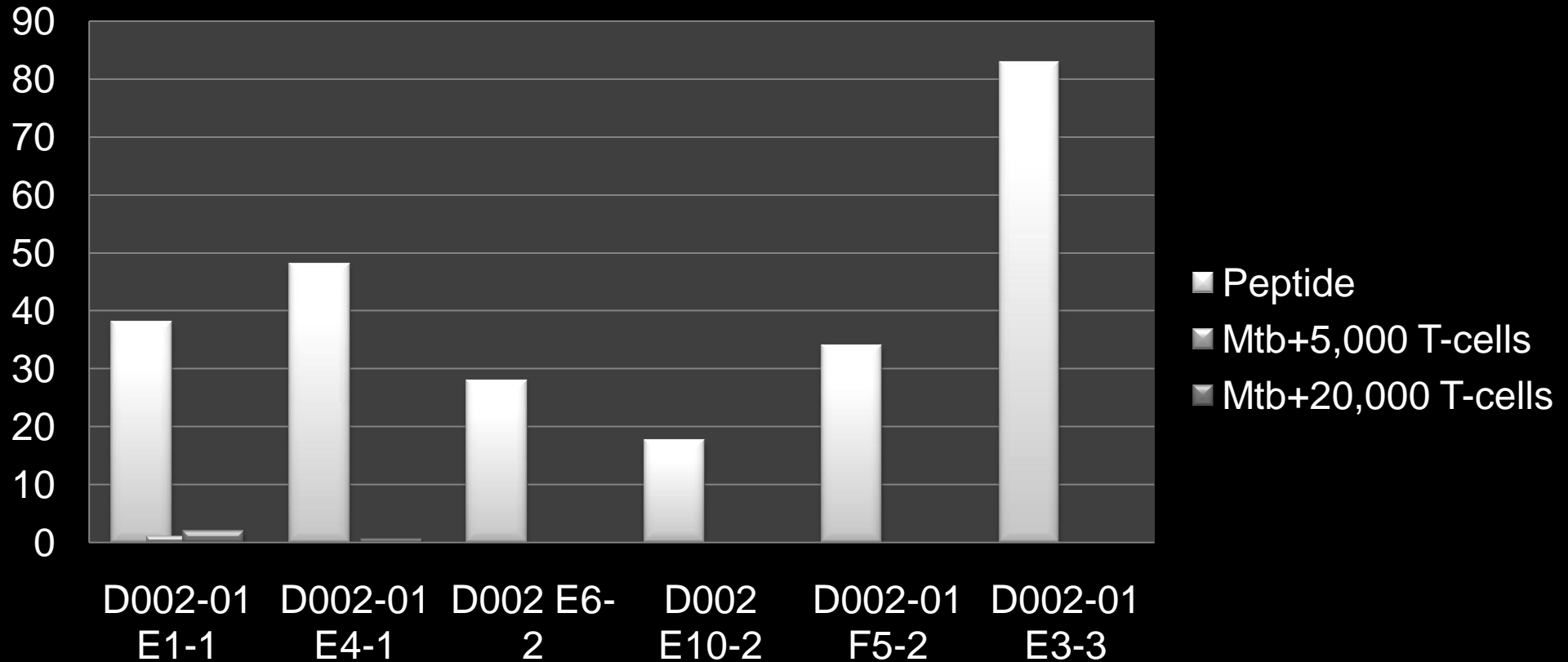


D002
E10-2



Evaluation of Clones

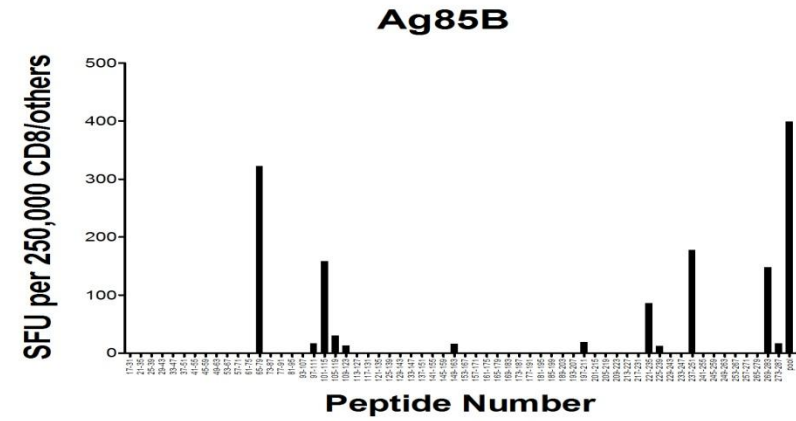
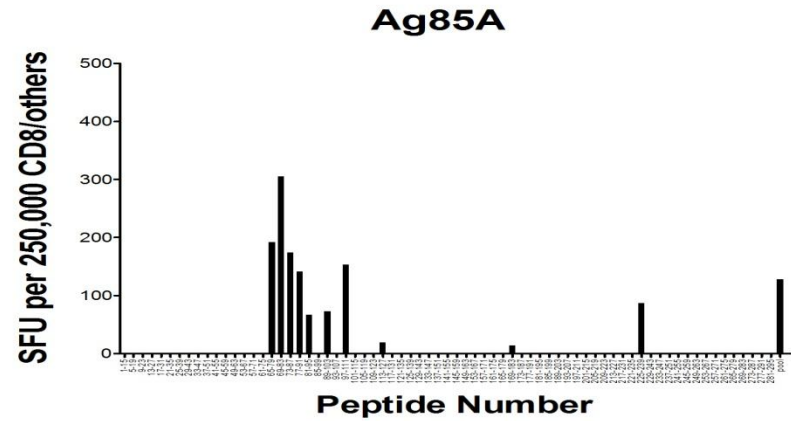
D002 Rescreen Results



T-cell Cloning – D008-03

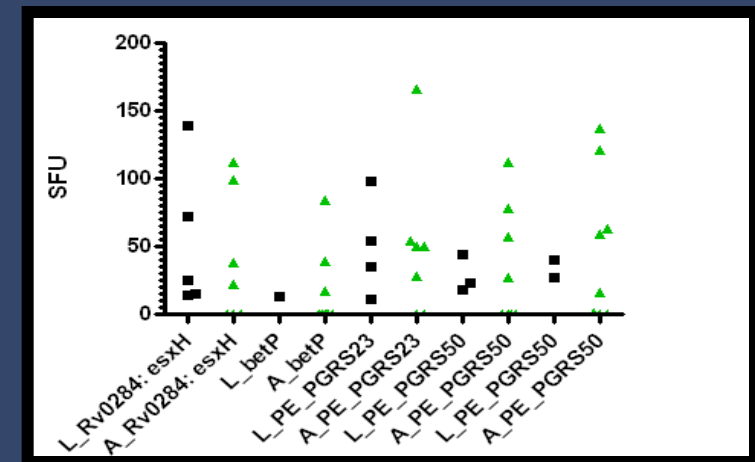
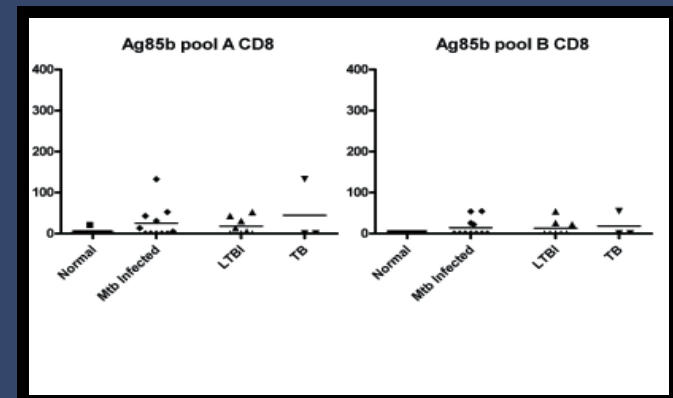
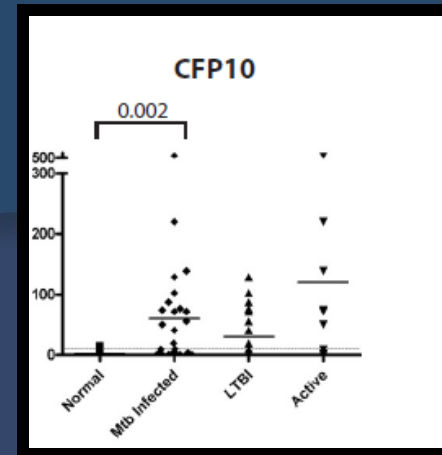
- Initial screen results:
 - 9 clones responded to Ag85A
 - 9 clones responded to Ag85B
 - 0 clones responded to Tb10.4
 - None responded to Mtb
 - Expansion and characterization of clones in progress

Ex-Vivo Analysis of Vaccine-Induced Epitopes



Food for Thought

- At least some vaccine induced epitopes are not displayed in an Mtb-infected DC.
- What governs immuno-dominance?
 - Abundance and stability of the antigen
 - Antigen processing and presentation
 - Host genetics
 - Status of the APC
 - PAMPS
 - Immuno-proteosome
 - Location of antigen processing
 - » HLA-B
- Can we induce a macrophage to display the epitope?
 - Induction of immuno-proteosome
- Can we skew a vaccine towards antigens/epitopes that are naturally processed and presented?
 - Additional antigens / antigenic regions?



Acknowledgements

- Portland VA Medical Center

Melanie Harriff
Lynne Swarbrick

- OHSU

Deborah Lewinsohn
Megan Null
Veena Rajaraman
Tomi Mori
Todd Vogt

- Collaborators

Henry Boom
Denise Johnson
Keith Chervenak
Dave Sherman
Karen Dobos
John Belisle
Lisa Wolf
Henry Boom
Roy Mugerwa
Harriett Myanja
Sarah Kiguli
Dennis Dobbs
Sarah Zalwango
Mary Nsereko
Payam Nahid
Alessandro Sette
John Altman
Alison Deckhut
Christine Sizemore

Ervina Winata
Joel Weekley

Melissa Nyendak
Meghan Cansler
Shannon McWeeney
Byun Park
Guanming Wu

CWRU
CWRU
CWRU
University of Washington
Colorado State University
Colorado State University
Colorado State University
Case Western Reserve University
Makerere University
Makerere University
Makerere University
TBRU
Makerere University
TBRU
UCSF
The La Jolla Institute for Allergy and Immunology
Emory University
NIH
NIH

Special thanks to:

Aeras / Crucell

Sean Bennett

Bruce McClain

Jerry Sadoff

Donata Sizemore

John Fulkerson

NIH

Christine Sizemore

Alison Deckhut

Timothy Gondre-Lewis

OHSU

Melissa Nyendak

Melissa Kumagi

Lynne Swarbrick

Ervina Winata

- Alteration of epitope recognition pattern in Ag85B and ESAT-6 has a profound influence on vaccine-induced protection against Mycobacterium tuberculosis
- Thomas Bennekov,
- Jes Dietrich,
- Ida Rosenkrands,
- Anette Stryhn,
- T. Mark Doherty,
- Peter Andersen Dr.
- Article first published online: 16 NOV 2006
- DOI: 10.1002/eji.200636128
- Copyright © 2006 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
- Issue
- European Journal of Immunology
- Volume 36, Issue 12, pages 3346–3355, December 2006
- Additional Information(Show All)
- Abstract
- To analyze the effect of vaccine delivery systems on antigen recognition and vaccine efficacy, we compared immune responses in mice immunized either with an adenovirus vector expressing a fusion of Ag85B and ESAT-6 or with the recombinant fusion protein in a liposomal adjuvant. Both vaccines induced high levels of antigen-specific IFN- γ production. The adjuvanted protein vaccine induced primarily a CD4 T cell response directed to the epitope Ag85B241–255 and gave efficient protection against subsequent Mycobacterium tuberculosis infection. In contrast, the adenoviral construct induced a strong CD8 response predominantly targeted to the epitope ESAT-615–29 and no significant protection against infection. Vaccination with the protein vaccine resulted in highly accelerated recall of Ag85B241–255-specific T cells immediately post M. tuberculosis challenge whereas the ESAT-615–29 epitope was barely recognized during infection. Delivery of the viral construct in cationic liposomes switched the immune response to a protective one dominated by CD4 T cells targeted to the Ag85B241–255 epitope. These data demonstrate that the nature of the T cell response to a vaccine antigen is more important than its magnitude with respect to protective efficacy and that vaccine-mediated changes in immunodominance can result in T cell responses of limited relevance during the natural infection.