

**Recognition and Control of Mtb Infected Cells:
From Basics to the Clinic
June 13 - 14, 2023
Virtual Workshop**

The role of cytotoxic lymphocytes in controlling TB

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Presenter Disclosures

David Lewinsohn, MD, PHD

Research Sponsorship

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Royalties and Patents

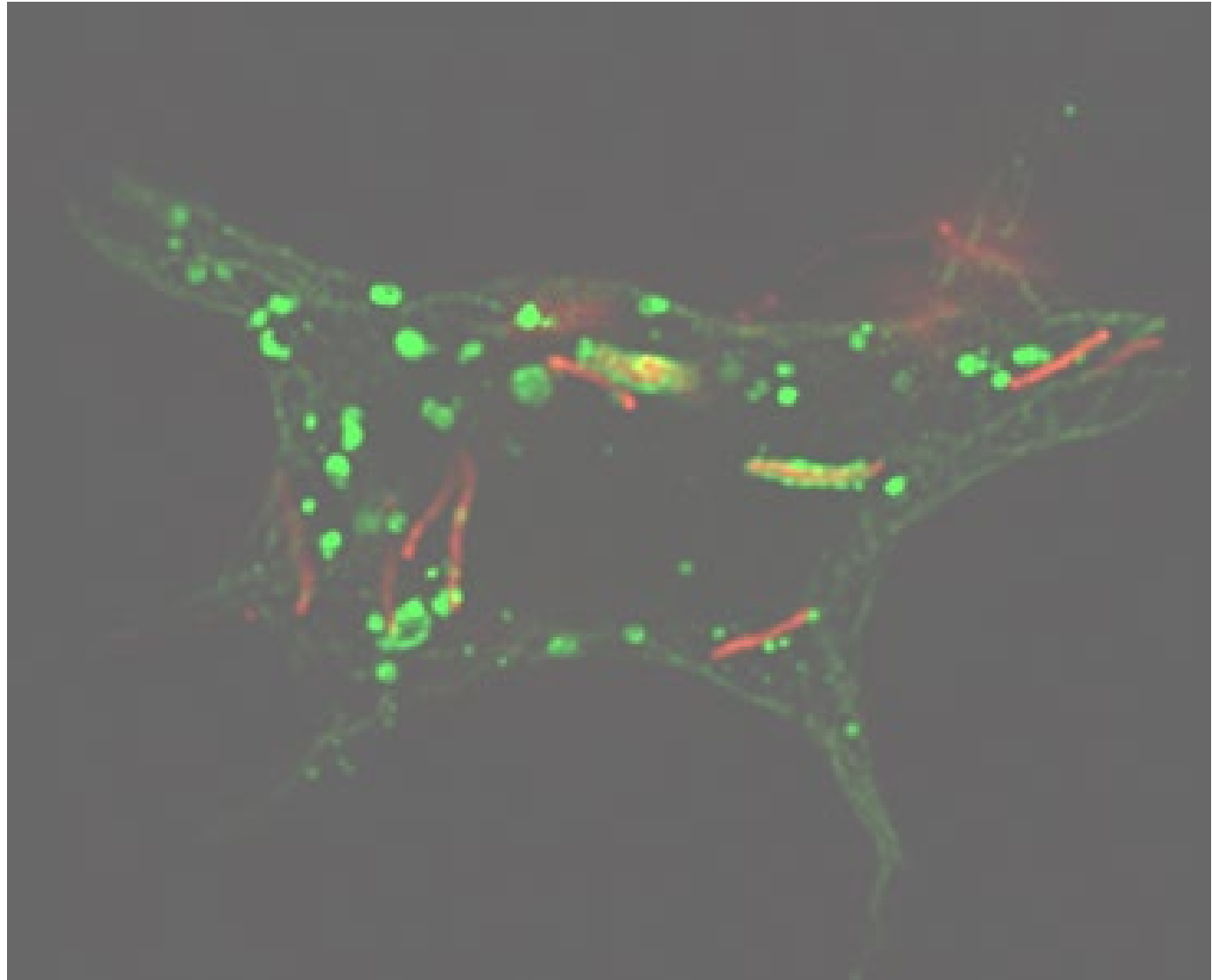
OHSU – CD8 Vaccines and Diagnostics

ViTi

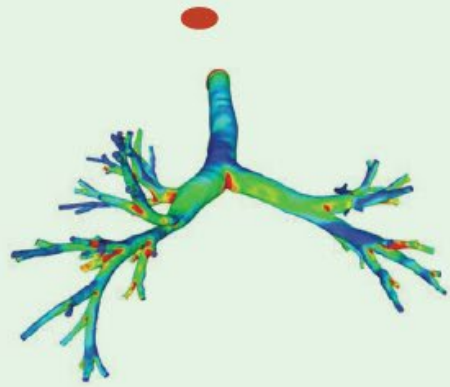
VIR

Detection of Intracellular Infection

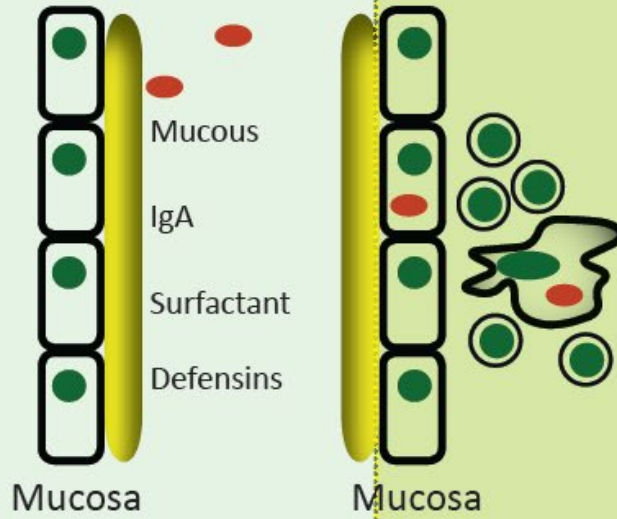
- Where is Mtb located in the human host?
 - The Lung
 - The granuloma?
 - Myeloid cells?
 - Epithelial cells?
 - Endothelial cells?
- How do T cells detect intracellular infection?
 - Presentation
 - Antigens
 - Tissue
 - Cell
 - HLA-II



Extracellular



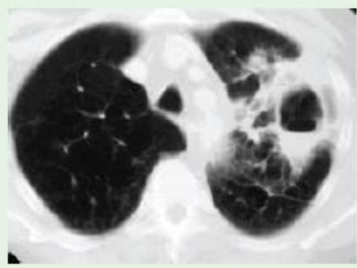
Airway



Mucosa

Mucosa

Cavity

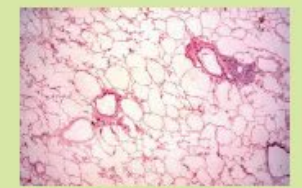


Defense Mechanisms

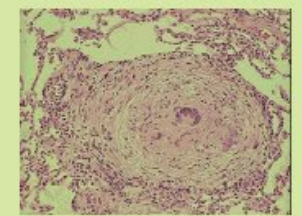
- B Cells
- Mucosal
- Neutrophils

Intracellular

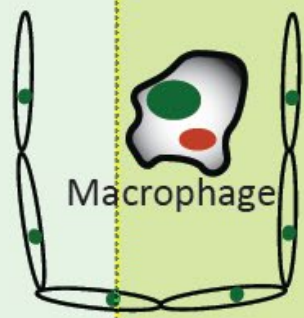
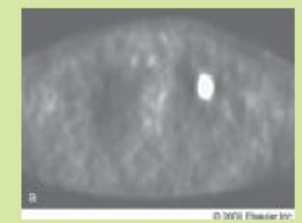
“Normal” Lung



Granuloma



Tuberculoma



Aiveolus

Defense Mechanisms

- CD4 T Cells (HLA-II only)
- CD8 T Cells
- HLA-Ia
- CD1a-c
- MR1
- HLA-E
- NKT Cells
- CD1d

The First Efficacy Trial of a TB Vaccine

- Temaris et al., Lancet 2013
- BCG followed by MVA85
 - 1399 MVA85
 - 1395 Placebo

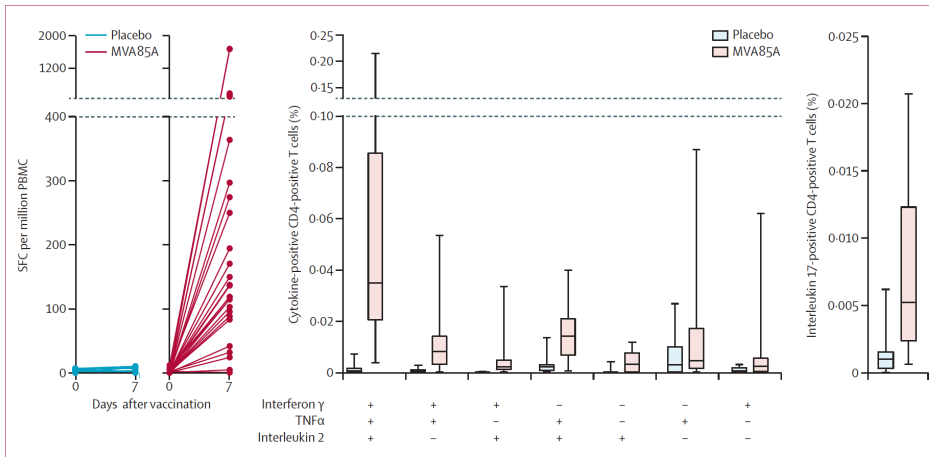
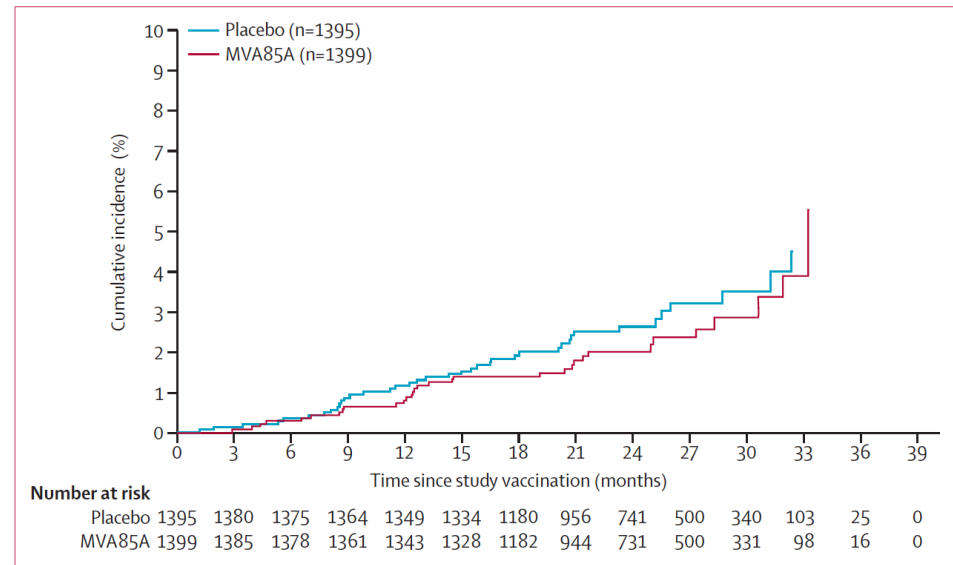


Figure 2: Vaccine immunogenicity
 (A) Frequencies of Ag85A-specific T cells measured by interferon- γ enzyme-linked immunosorbent spot assay in infants in study group 2 (27 infants in the MVA85A group and 27 infants in the placebo group) before administration of placebo or MVA85A (day 0) and 7 days after vaccination. (B) Frequencies of cytokine-expressing Ag85A-specific Th1 (CD4-positive T cells expressing IFN- γ , TNF α , or interleukin 2) and (C) frequencies of Ag85A-specific Th17 (CD4-positive T cells

	Placebo (n=1395)	MVA85A (n=1399)	Vaccine efficacy
Endpoint 1 (primary efficacy endpoint)	39 (3%)	32 (2%)	17.3% (-31.9 to 48.2)
Endpoint 2 (exploratory efficacy endpoint)	52 (4%)	55 (4%)	-6.9% (-56.1 to 26.9)
Endpoint 3 (exploratory efficacy endpoint)	177 (13%)	196 (14%)	-12.1% (-37.4 to 8.5)

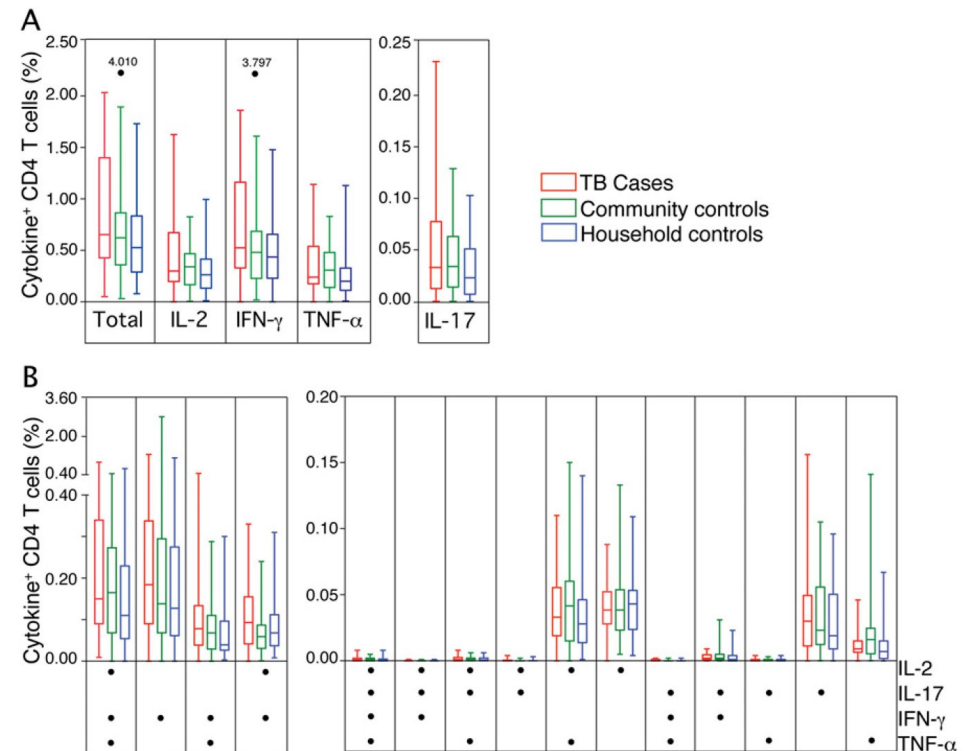
Data are n (%) or % (95% CI). Participants with more than one diagnosis were analysed in each level of diagnosis attained. Vaccine efficacy and corresponding 95% CI was estimated with the Cox regression model (1-estimated hazard ratio).

Table 2: Primary and secondary efficacy endpoints

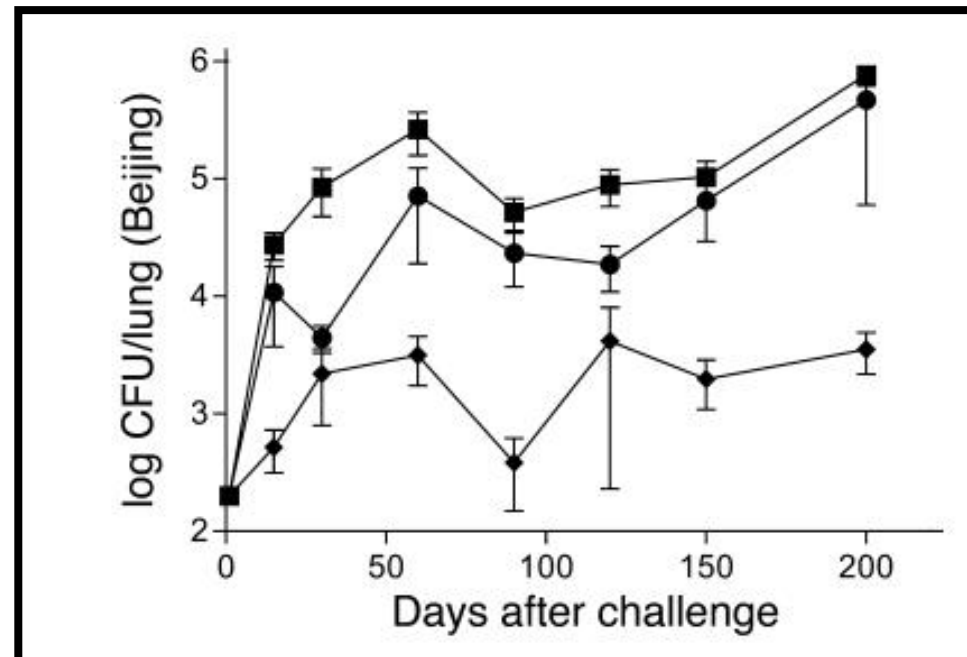
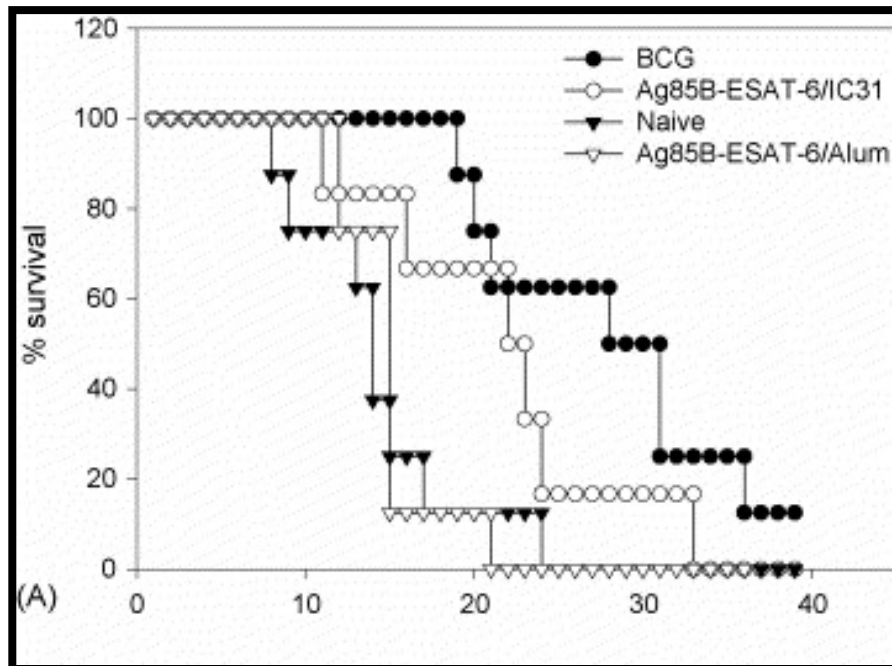


What Happened?

- CD4 Responses not Important
- We Have the Wrong Antigens
- We Have the Wrong T Cells
- We need Antibodies

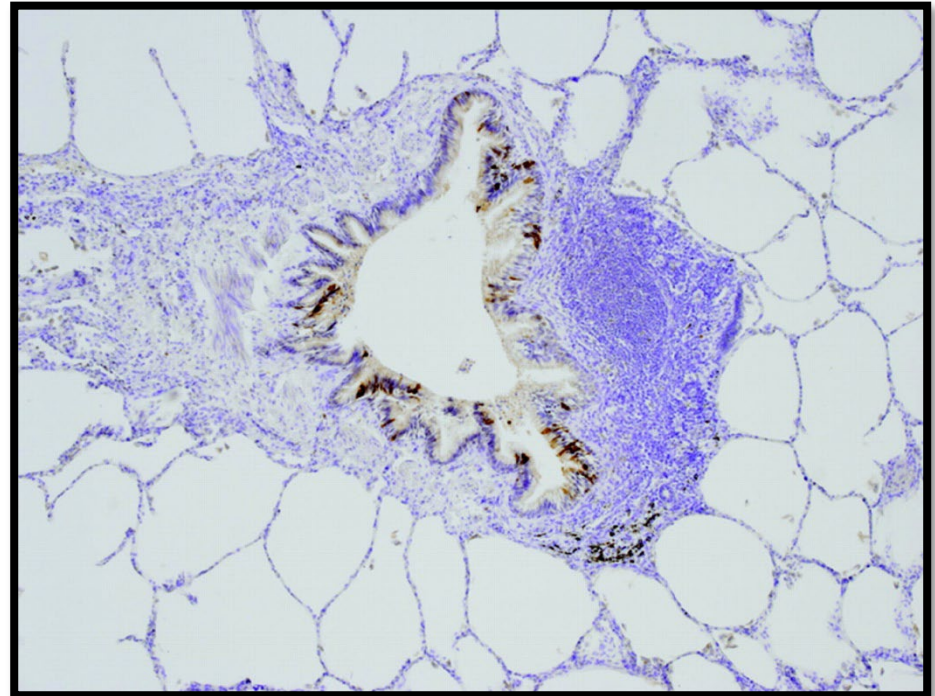


The Ah Ha Experience?

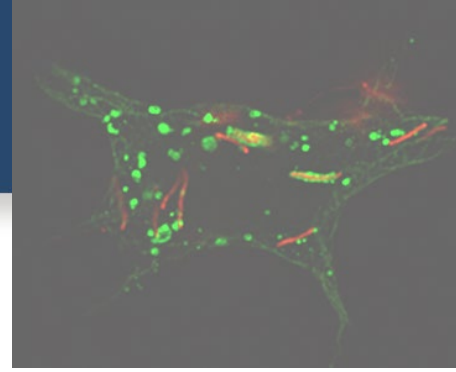


The Host: Immune Defenses in the Airway

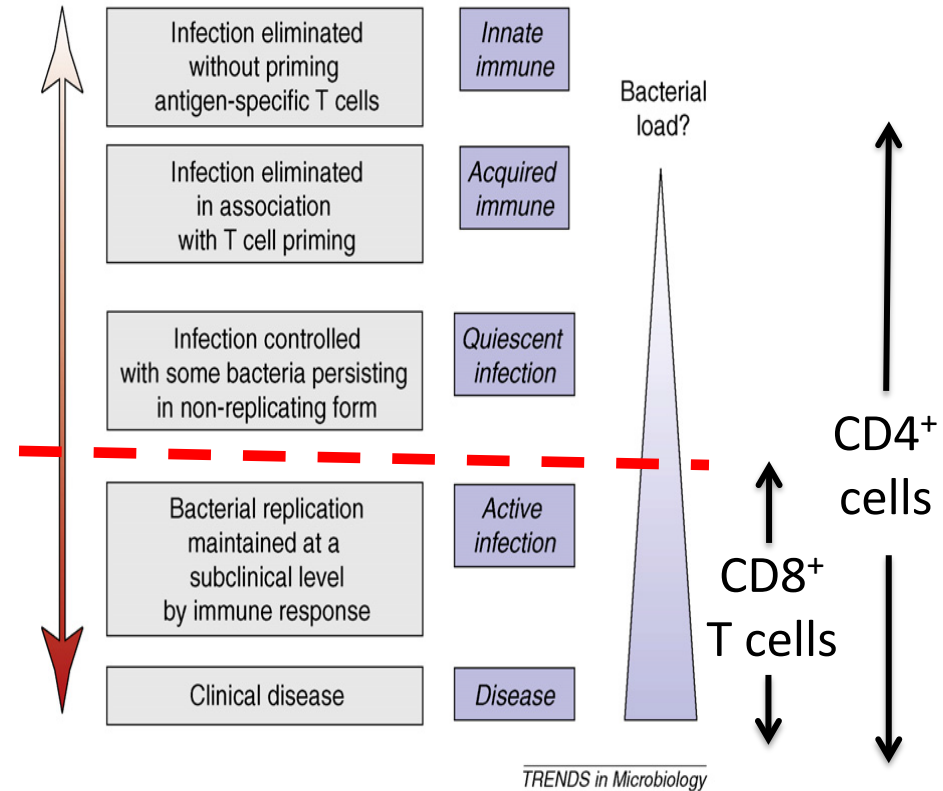
- Advantages to recognizing Mtb in the airway
 - Early control
 - Facilitate the acquisition of adaptive immunity



Unique functions of CD8⁺ T cells

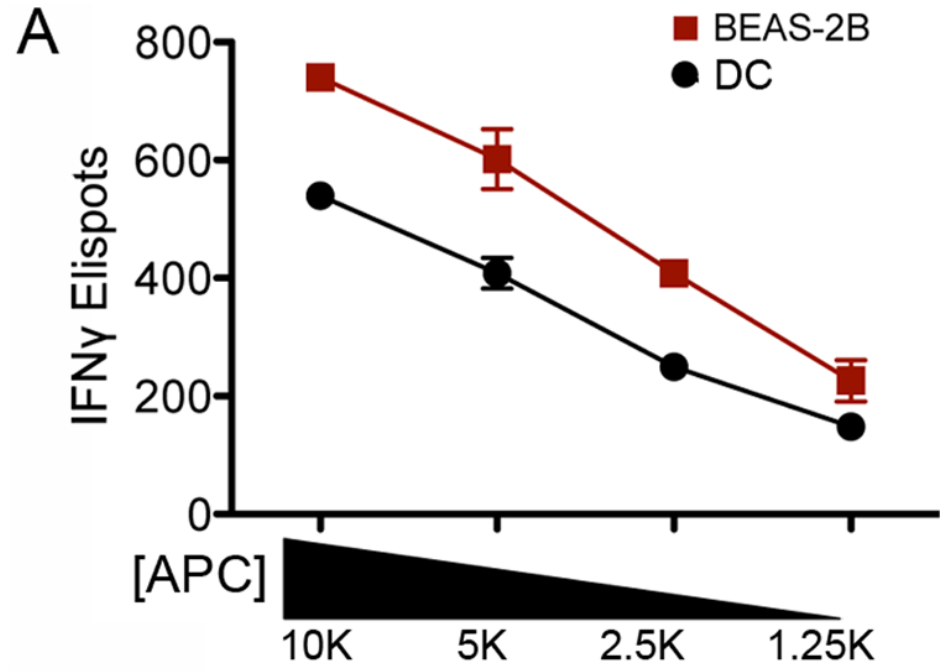
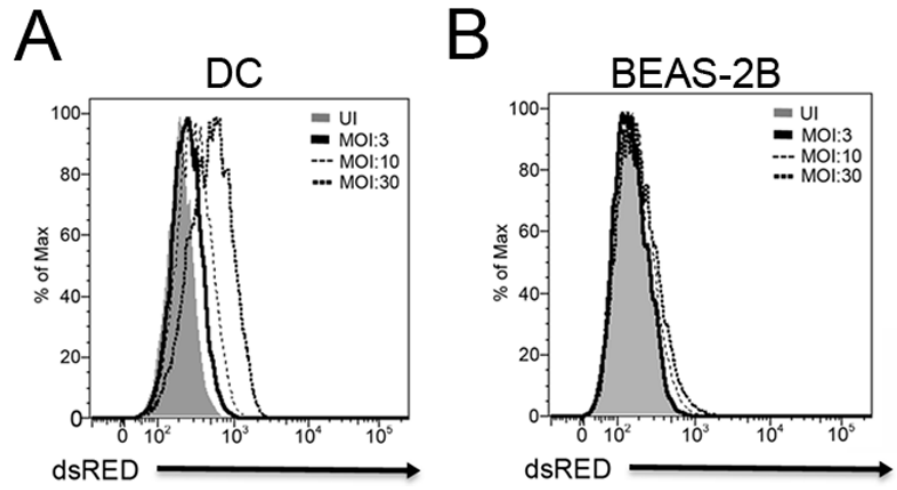
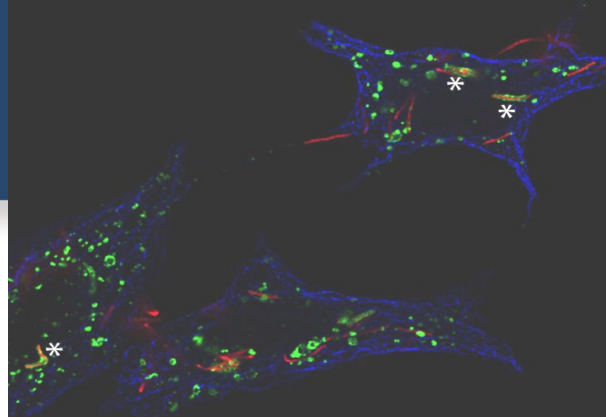


- Recognition of MHC Class II negative cells
- Preferentially recognize heavily infected cells
- Discern bacterial burden



Adapted from Young DB et al.,
Trends Microbiol 2009

Human Lung Epithelial Cells are Efficiently Recognized by CD8 T Cells

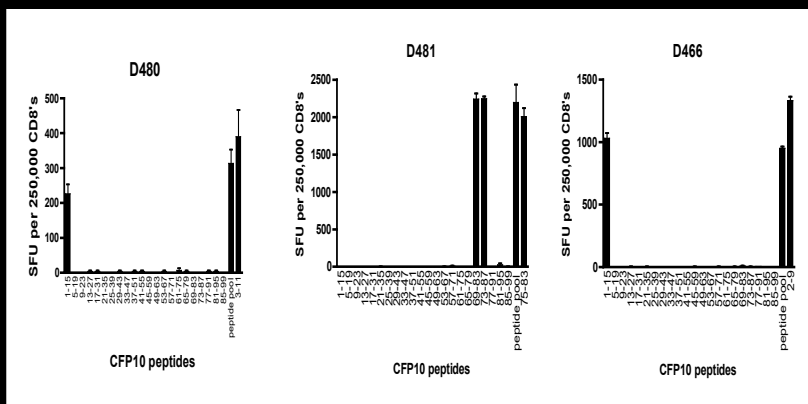
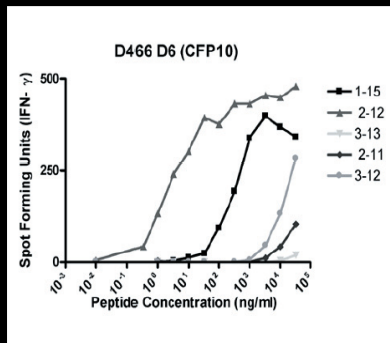


Harriff et al., PLOS one 2014

Human CD8+ T Cells Recognize the Infected Cell



- HLA-B restriction is common
- Epitopes are often 10 or 11aa in length
- Immuno-dominance
- Novel antigens
 - EsxJ Family
 - PE9
 - PE/PGRS 42
- New HLA prediction tools accurate



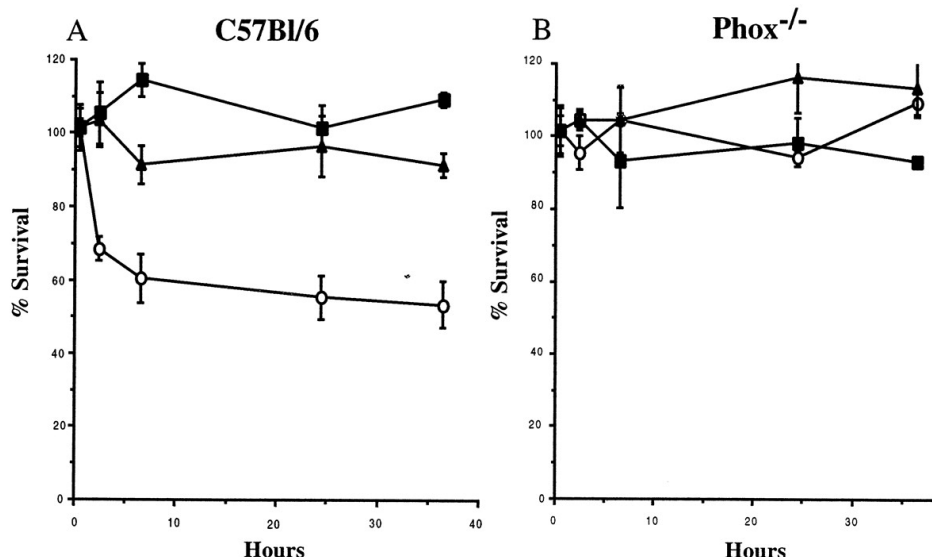
Donor	Mtb Specific T cells ^a	Clone ^b	Gene ^c	HLA-Restricting Allele	Epitope Location	Epitope Sequence	Epitope Specific T cells ^a	MHC Binding Affinity (IC50 nM)	Vbeta region
160	61 ^d	D160 1-1B ^d (0)	CFP10	B44	2-11	AEMKTDAAATL	360	38	
160	61 ^d	D160 1-6F ^d (0)	CFP10	B14	85-94	RADEEQQQAL	120	NA	
432	216	D432 H12 (2)	CFP10	B3514	49-58	TAAQAAVVRF	258	2011 ^e	5.3
466	473	D466 A10 (10)	CFP10	B4501	2-9	AEMKTDAA	2458	48	IND
466	473	D466 D6 (1)	CFP10	B4501	2-12	AEMKTDAAATLA	1993	6.2	22
481	405	D481 C10 (10)	CFP10	B1502	75-83	NIRQAGVQY	1715	14 ^f	9
481	405	D481 C11 (1)	CFP10	B1502	75-83	NIRQAGVQY	1715	14 ^f	13.6
480	598	D480 F6 (6)	CFP10	B0801	3-11	EMKTDAAATL	387	79	13.1
571	TBD	D571 B12 (3)	CFP10	B4402	2-11	AEMKTDAAATL	31	38	IND
571	TBD	D571 E9 (4)	CFP10	B4402	2-11	AEMKTDAAATL	31	38	14
504	126	D504 E4 (1)	Mtb9.8	A0201	3-11	LLDAHIPQL	<10	0.39	8
454	138	D454 B10 (1)	Mtb9.8	B0801	53-61	AAHARFVAA	88	0.22	IND
454	138	D454 H1-2 (1)	Mtb8.4	B1501	5-15	AVINTTCNYGQ	24	10	7.1
432	216	D432 A3 (2)	Mtb 8.4	B3514	32-40	ASFVAQSYL	210	127 ^e	14
443	53	D443 H9 (1)	Ag85B	TBD	144-153	ELPQWLSANR	<10	NA	22

How Might the Host Kill Mtb?

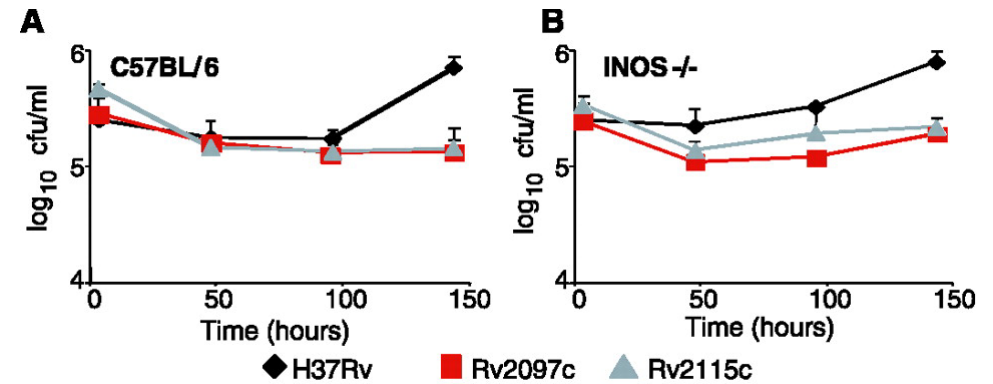
- Activation of macrophages
 - IFN- γ ; TNF- α
 - NO; H₂O₂
 - Acidification + proteases
- Modification of the intracellular environment
 - Induction of apoptosis
 - Perforin & granzyme
 - Introduction of anti-mycobacterial peptides
 - Granulysin
 - Induction of autophagy
 - IFN- γ

Mtb: Resistance to H2O2 and Nitric Oxide

H2O2



Nitric Oxide



The Proteasome of *Mycobacterium tuberculosis* Is Required for Resistance to Nitric Oxide

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Nadine Weich,² Carl F. Nathan^{1,3*}

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Cu,Zn Superoxide Dismutase of *Mycobacterium tuberculosis* Contributes to Survival in Activated Macrophages That Are Generating an Oxidative Burst

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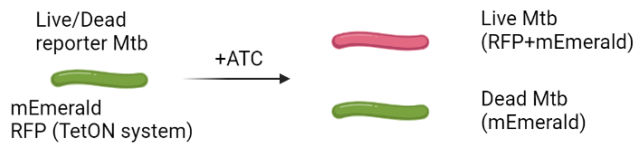
Received 15 December 2000/Returned for modification 15 January 2001/Accepted 1 May 2001

INFECTION AND IMMUNITY, Aug. 2001, p. 4980-4987
0019-9567/01/\$04.00+0 DOI: 10.1128/IAI.69.8.4980-4987.2001
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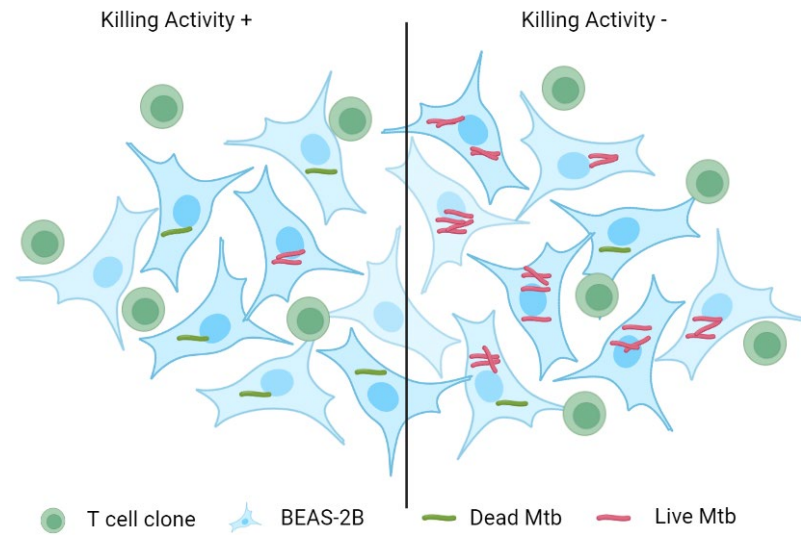
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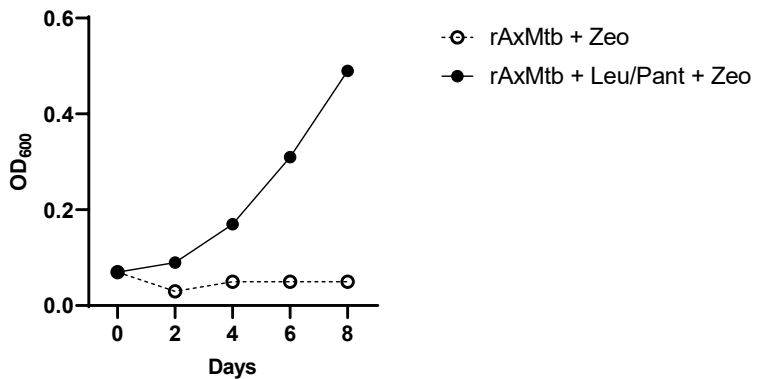
Live Dead reporter Mtb strain



Sensitive Intracellular Mtb Killing Assay



rAuxotroph Mtb ($\Delta leuD\Delta panCD$)

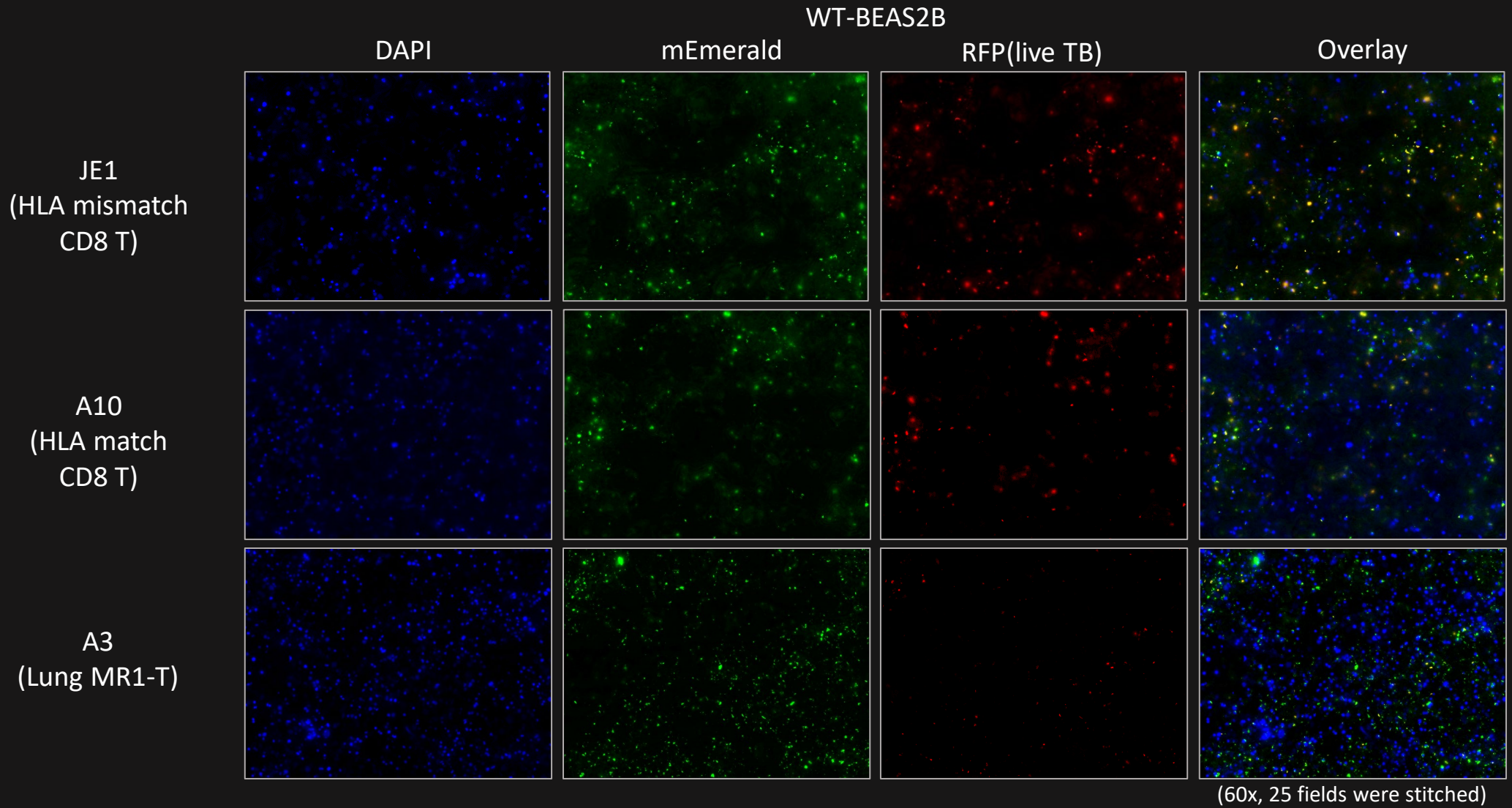


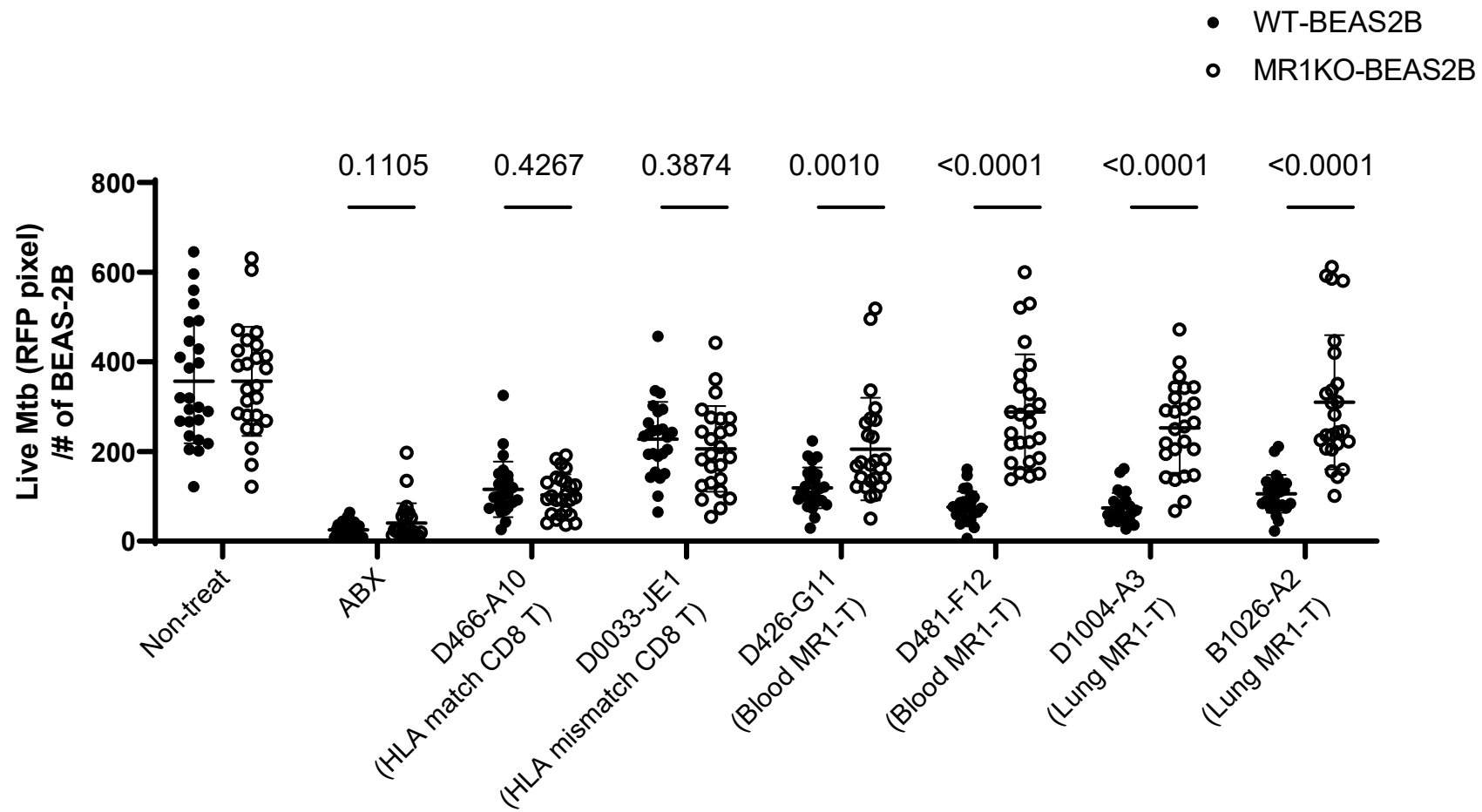
Courtesy
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Sam Behar
Bryan Bryson



Shogo Soma,
DVM PhD

Differential T Cell Dependent Killing





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