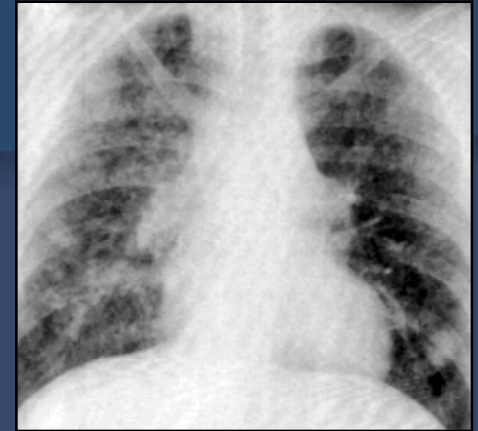


*2nd Global Symposium on IGRAs :
San Francisco, CA*



Rethinking the Natural History of Infection with Mtb

David Lewinsohn, MD, PhD
Pulmonary & Critical Care Medicine

Portland VA Medical Center
Oregon Health & Sciences University

Natural History of TB



Possible Outcomes

Active Tuberculosis

Latent Tuberculosis

Clearance(?)



"Sputum induction is putting a patient and Ann Elarth together in a well-ventilated room."

Charlie Nolan



Rethinking Latent TB



Rethinking Latent TB

- Recently exposed?
- BCG vaccinated?
- INH toxicity?
- Benefit from therapy?

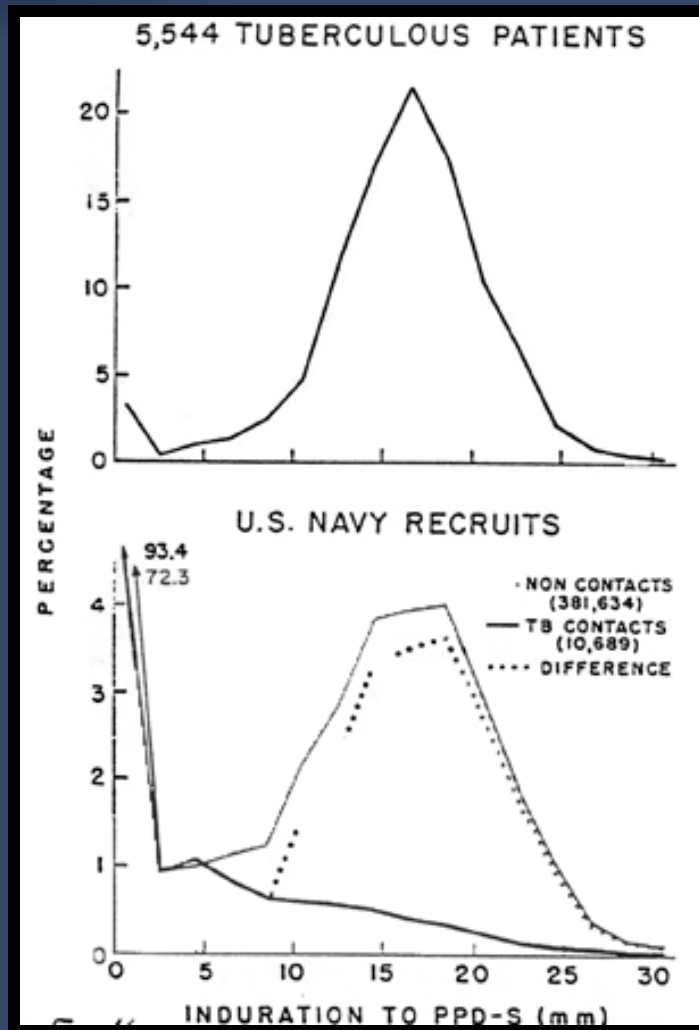


Rethinking Latent TB

- Is there evidence for transient exposure?
- What is persistent TB?
- Can we discern recent infection or re-infection?



The Natural History of Tuberculosis: Current Status



TST

- Associated with Exposure to Mtb
- Conversion associated with risk of disease

Proposed Classification of Infection with Mtb (TBI)

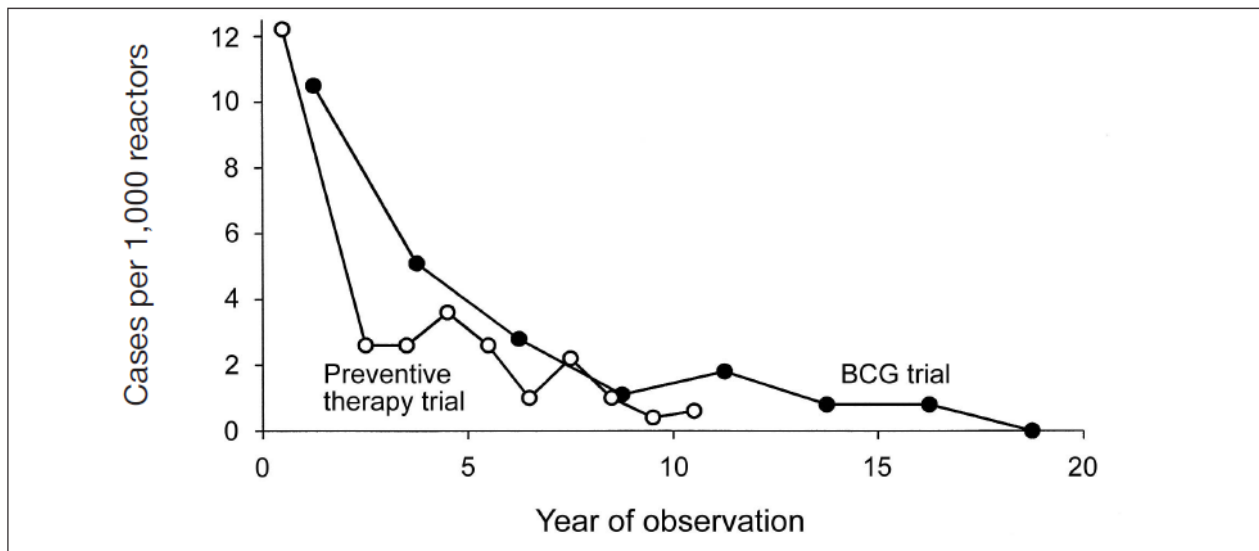


Figure 52. Incidence of tuberculosis among household contacts receiving placebo, compared to household contacts receiving preventive chemotherapy with isoniazid [134] and number of tuberculosis cases among unvaccinated persons with large tuberculin skin test reactions by time interval after beginning of observation in a controlled clinical trial on BCG vaccination in Great Britain [135].

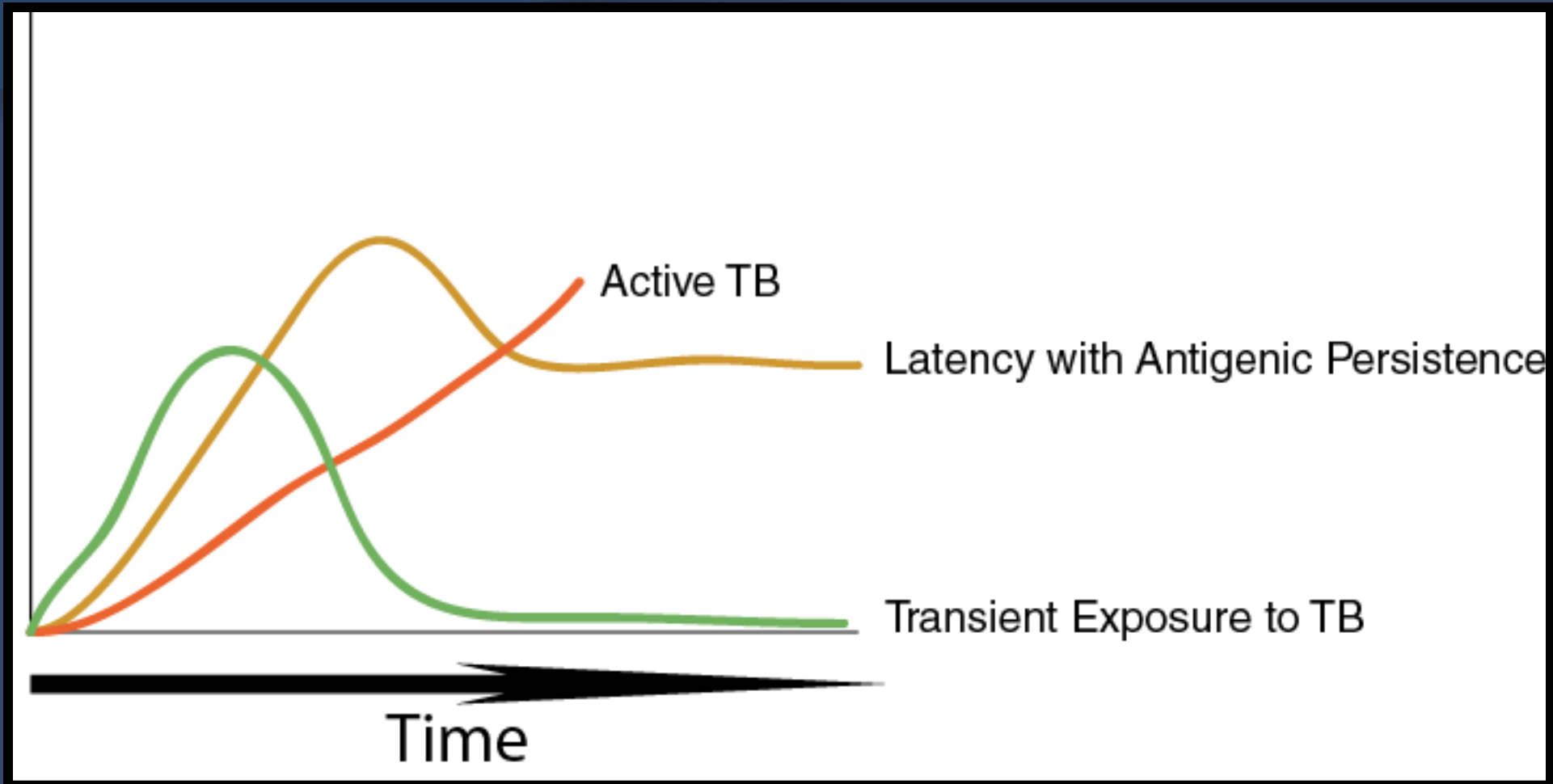
- Risk highest shortly after infection
- Late disease might reflect re-exposures
- TST poor at identifying those at risk

The Natural History of Tuberculosis: Revisited

IFN- γ

- Associated with Exposure to Mtb
- Quantitative
 - Remote vs Recent Infection
 - Reflection Antigen Load
 - Natural variations in antigen load over time
 - Disease treatment
 - New exposures
 - Risk for reactivation

Model of Memory: Possible Outcomes



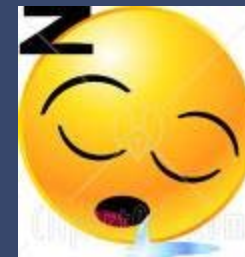
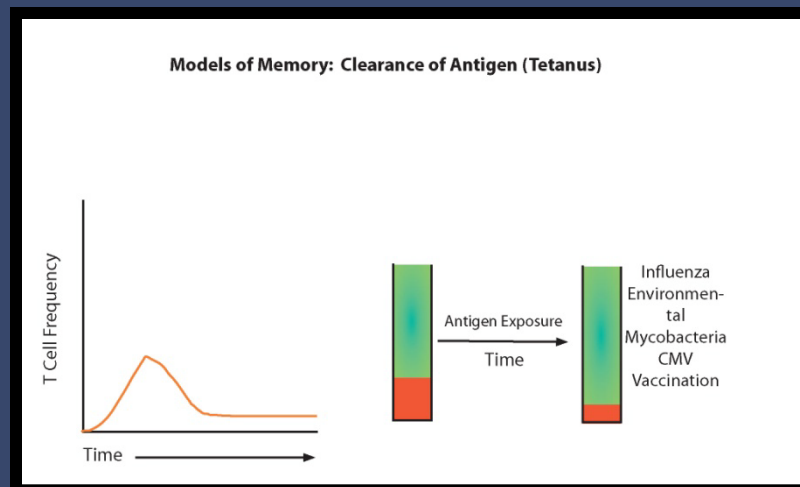
Food For Thought:

T-Cell Based Diagnosis of Latent Tuberculosis Infection in Resource-Limited Settings, Geneva 2006

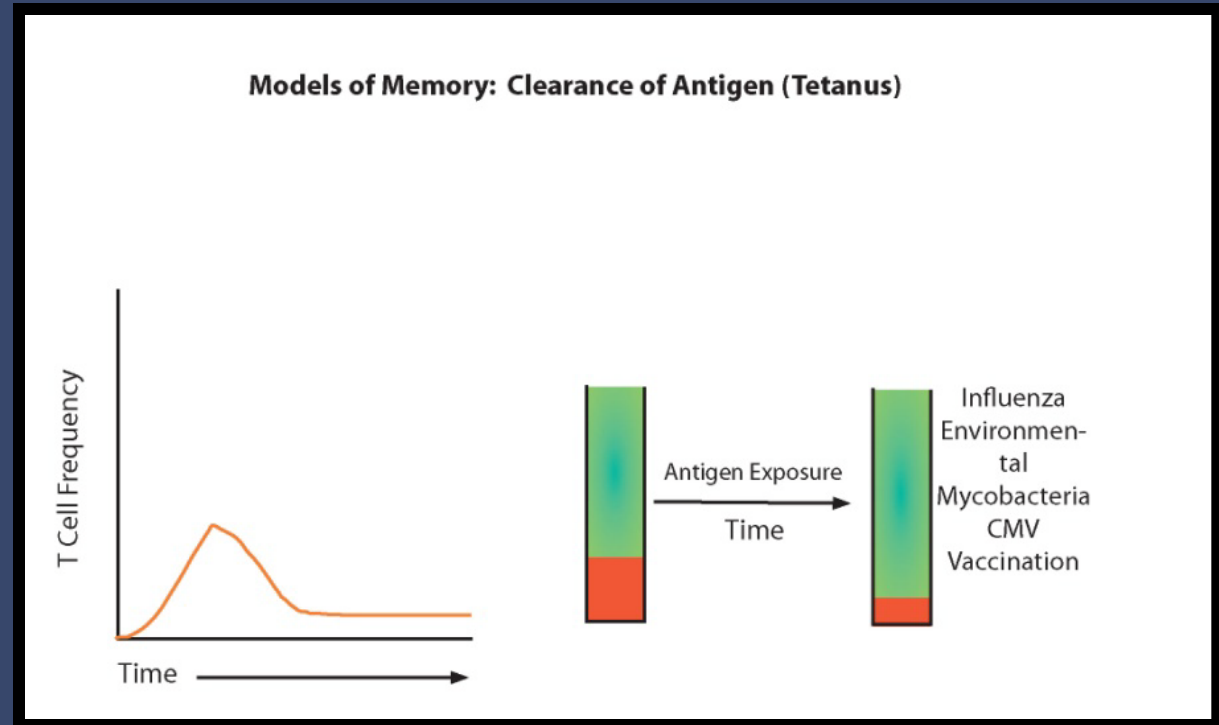
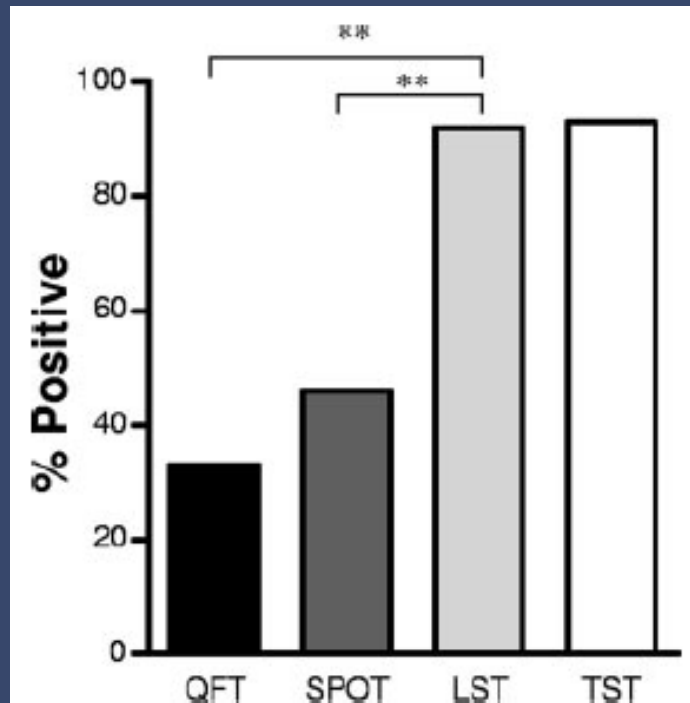
- In longitudinal testing, a dramatic rise in IFN- γ will indicate either recent exposure to Mtb or reactivation.
 - Pediatrics. 2007 Jan;119(1):e1-5. T-cell-based diagnosis of neonatal multidrug-resistant latent tuberculosis infection.
Richeldi L, Ewer K, Losi M, Bergamini BM, Millington K, Fabbri LM, Lalvani A.
 - INT J TUBERC LUNG DIS. 2007 11(3):350–352. Rising ELISPOT count prior to the onset of symptoms of full-blown tuberculosis disease
I. M. O. Adetifa, R. Brookes, M. D. Lugos, B. C. de Jong, M. Antonio, R. A. Adegbola, P. C. Hill

Food For Thought: Predictions: T-Cell Based Diagnosis of Latent Tuberculosis Infection in Resource-Limited Settings, Geneva 2006

- A negative IFN- γ assay may not distinguish remote exposure from no exposure.
 - Some will be transiently exposed, and will develop TEM/TCM (like Tetanus).
 - The duration of these responses will depend on the environment.
 - Low risk for reactivation



Food For Thought: Predictions: T-Cell Based Diagnosis of Latent Tuberculosis Infection in Resource-Limited Settings, Geneva 2006



Leyton et al., *Clinical and Vaccine Immunology*, 2007

Food For Thought: Predictions: T-Cell Based Diagnosis of Latent Tuberculosis Infection in Resource-Limited Settings, Geneva 2006

- Alternate explanations for IGRA / TST discordance:
 - BCG
 - Environmental mycobacteria
 - Prevalence of exposure to Mtb
 - DTH vs PBMC

	TST	IFN
Cell Types	CD4 CD8 Basophils DC and Macrophages	CD4 (CD8)
Cytokines	IL-4, IFN- γ TNF- α , IL-10, IL-12, G-CSF,	IFN- γ
Associated with Protective Immunity	No	Yes
Timing	2-5 Days	Short Term
Homing Phenotype	Skin	Polymorphic

Food For Thought: Predictions: T-Cell Based Diagnosis of Latent Tuberculosis Infection in Resource-Limited Settings, Geneva 2006

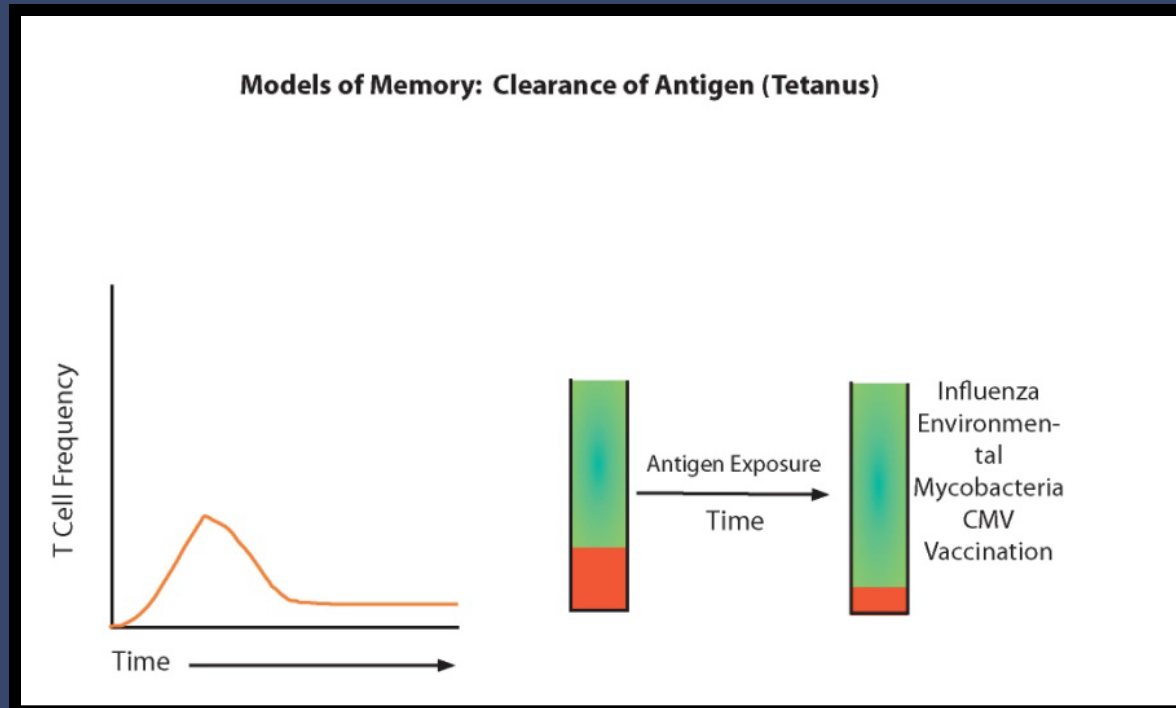
A negative IFN- γ assay may not distinguish remote exposure from no exposure.

– Low risk for reactivation

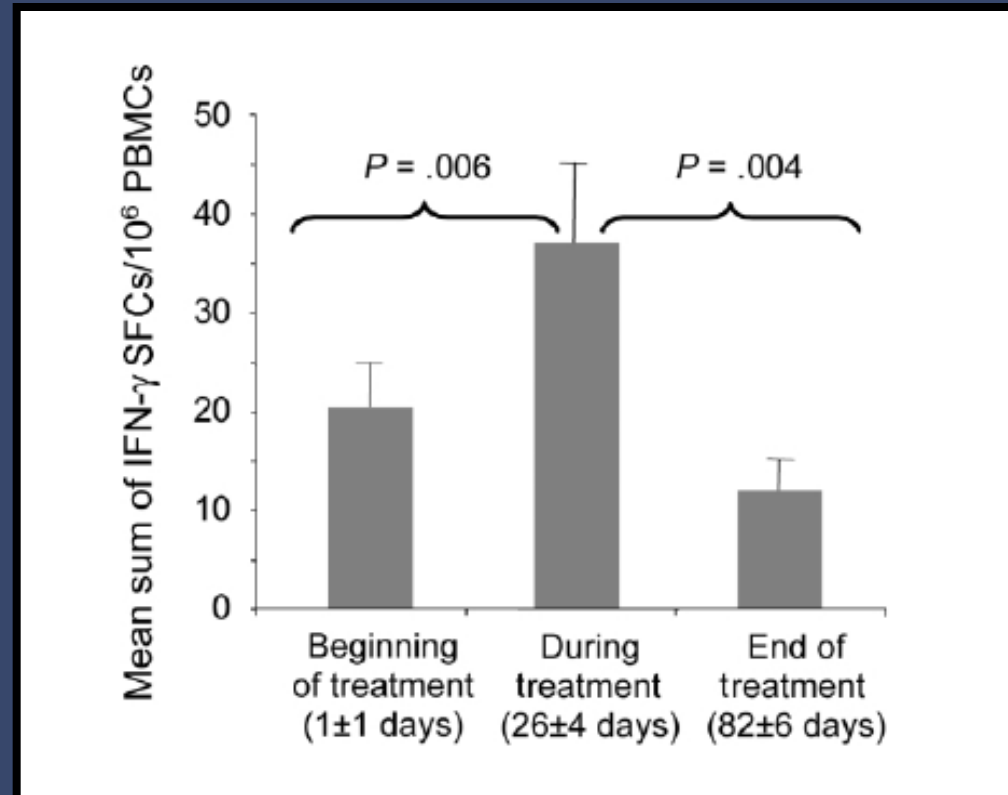
- Am J Respir Crit Care Med. 2008 May 15;177(10):1164-70. Epub 2008 Feb 14. Predictive value of a whole blood IFN-gamma assay for the development of active tuberculosis disease after recent infection with Mycobacterium tuberculosis. Diel R, Loddenkemper R, Meywald-Walter K, Niemann S, Nienhaus A.
- Ann Intern Med. 2008 Dec 2;149(11):777-87. Epub 2008 Oct 20. Prognostic value of a T-cell-based, interferon-gamma biomarker in children with tuberculosis contact. Bakir M, Millington KA, Soysal A, Deeks JJ, Efee S, Aslan Y, Dosanjh DP, Lalvani A.

Food For Thought: Predictions: T-Cell Based Diagnosis of Latent Tuberculosis Infection in Resource-Limited Settings, Geneva 2006

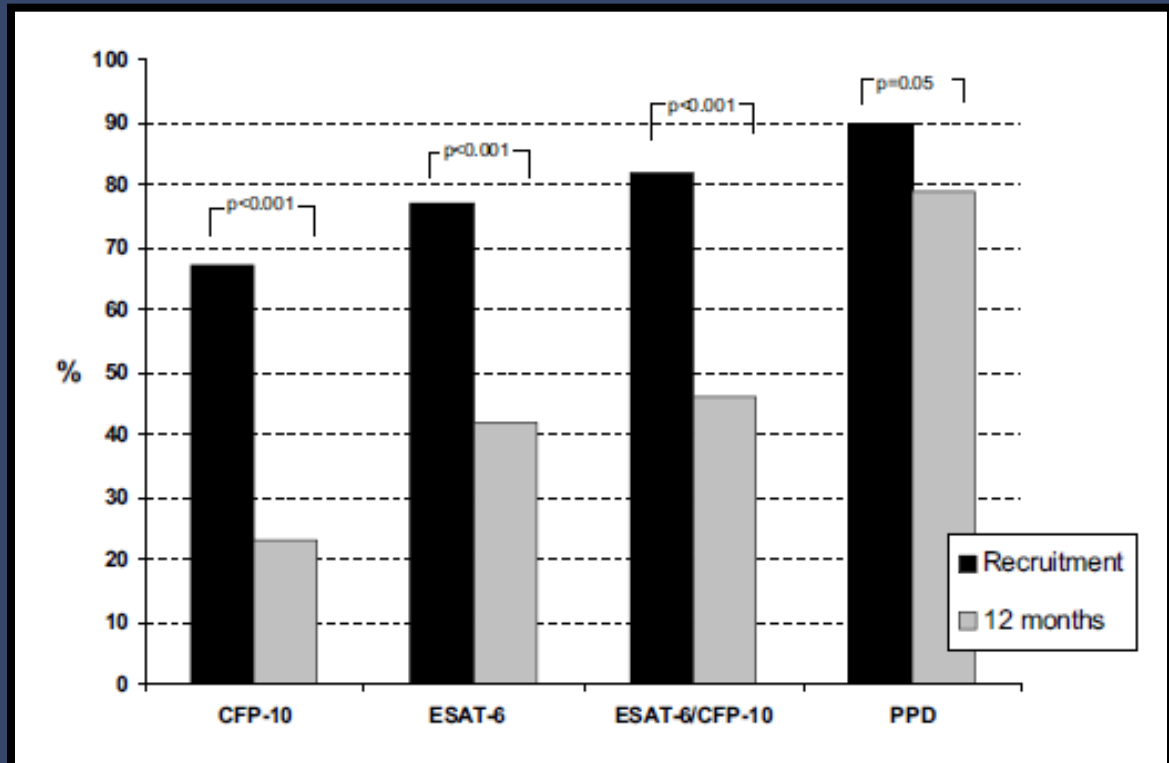
- The IFN- γ responses will decrease with time following effective treatment



Longitudinal
assessment of
33 subjects
treated for LTBI

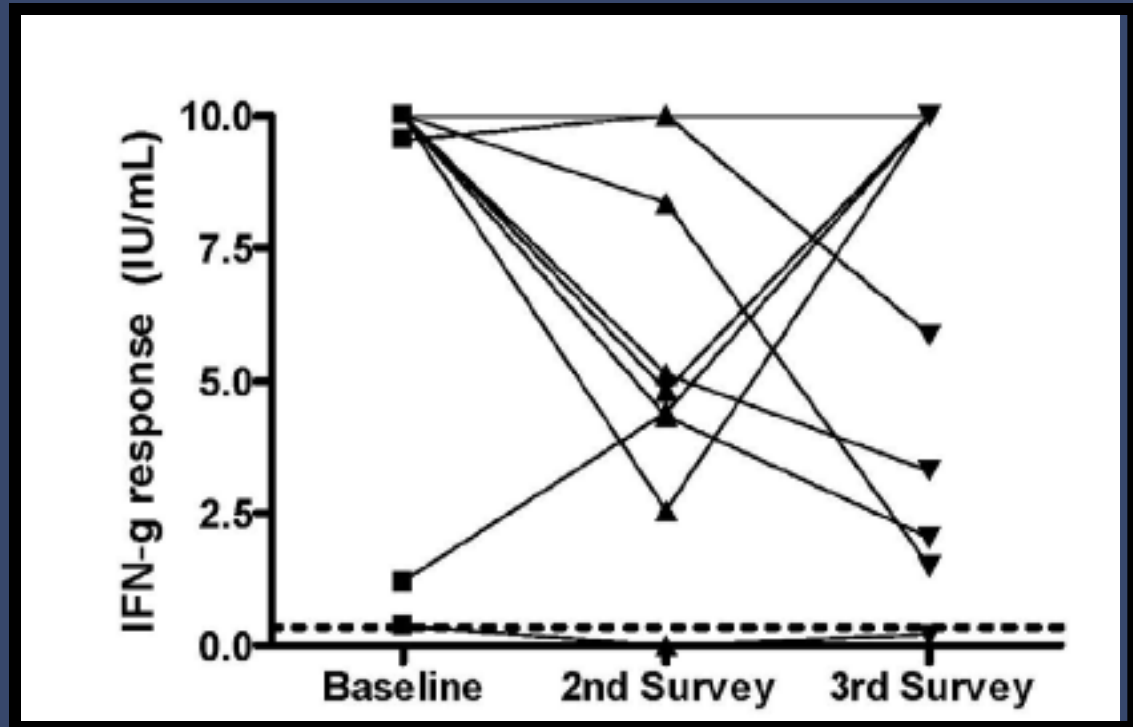


- ELISPOT test at diagnosis and 12 months later in 89 patients.
- 82% ESAT-6 or CFP-10 (EC) ELISPOT positive
- 55% EC ELISPOT negative at 12 months



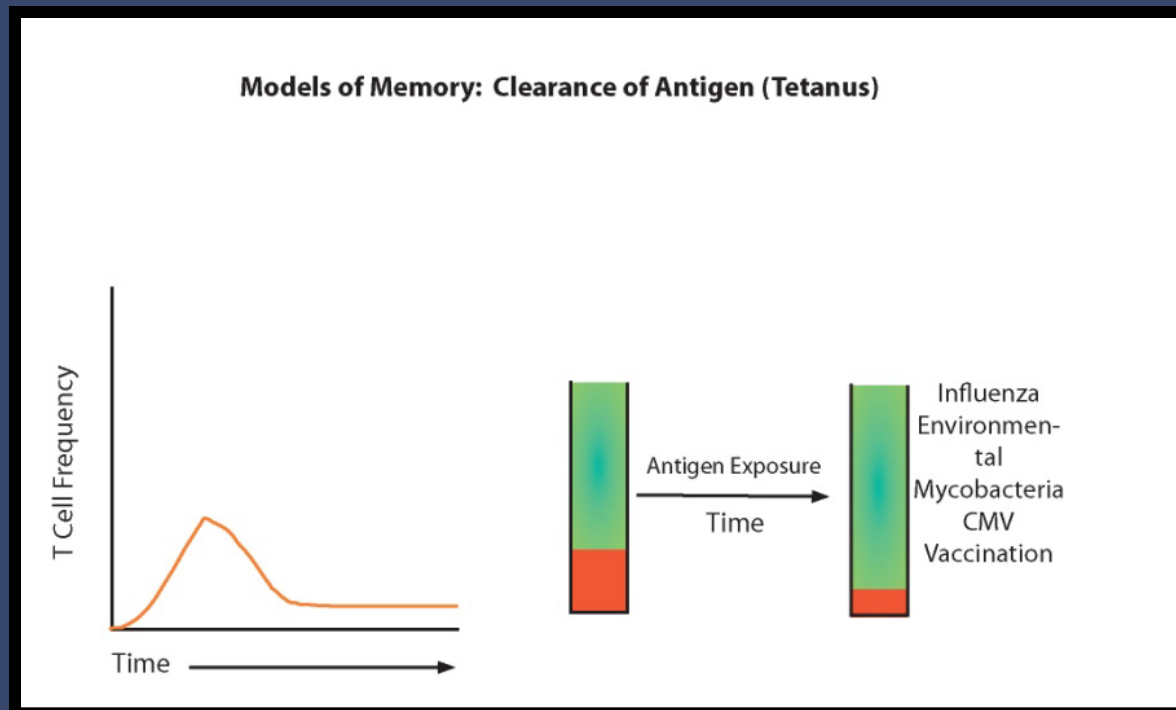
Pai et al., Journal of Occupational Medicine and Toxicology 2006, 1:7

Prospective study of 10 HCW receiving IHN prophylaxis



Food For Thought: Predictions: T-Cell Based Diagnosis of Latent Tuberculosis Infection in Resource-Limited Settings, Geneva 2006

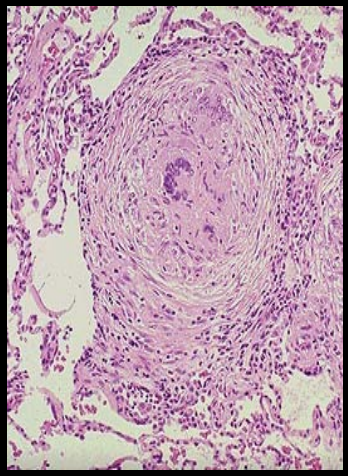
- The IFN- γ responses will decrease with time following effective treatment.
 - Maybe



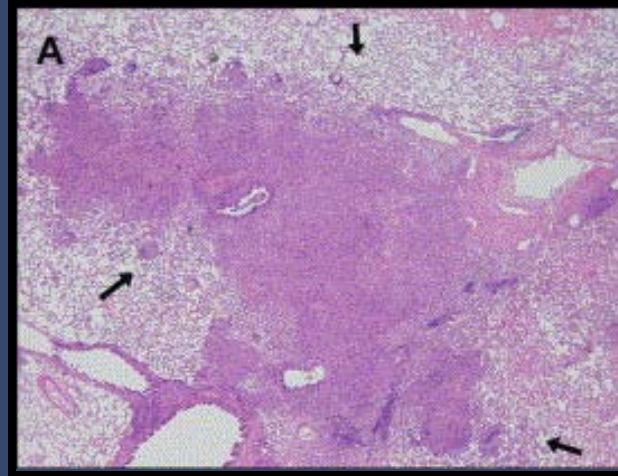
Understanding T Cell Memory

- What about persistent infection?

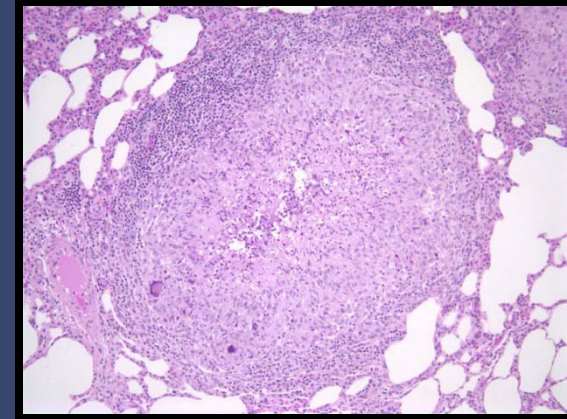
Animal Models



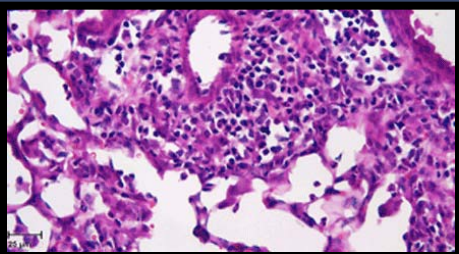
Human



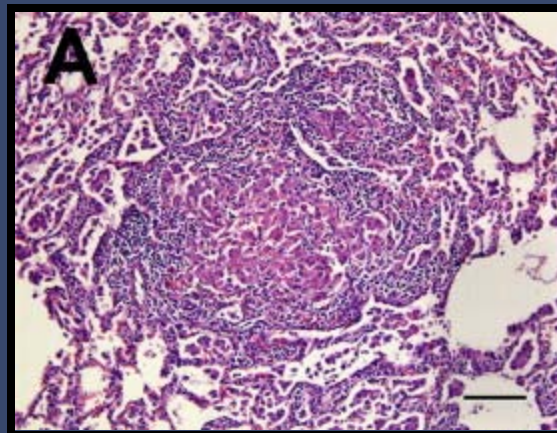
Guinea Pig



Rhesus Macaque



Mouse



Rabbit

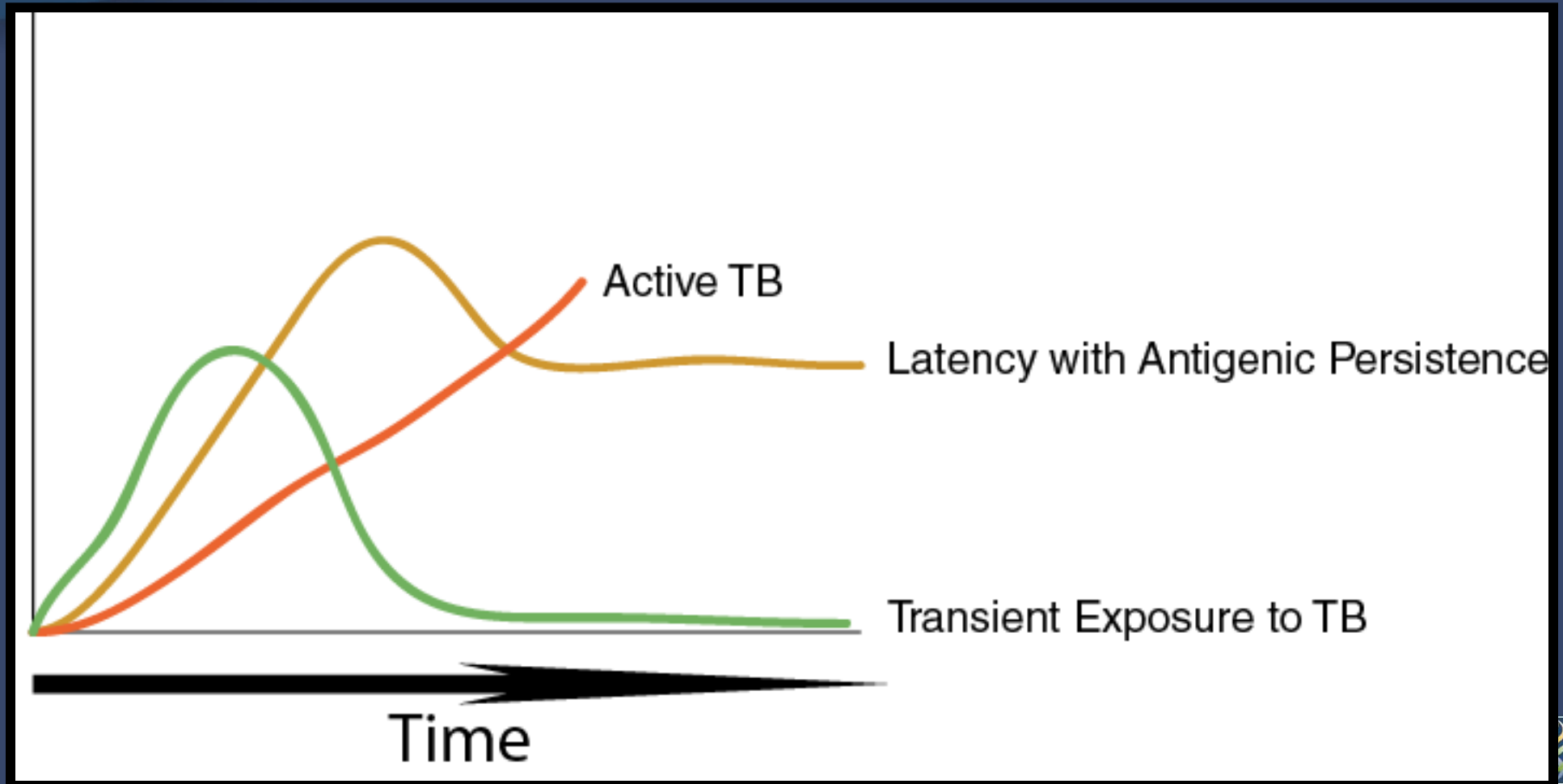
Persistent TB Infection

- 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

Persistent TB Infection

- Clinical relevance
 - Immunosuppression
 - Organ transplantation
- What is the best diagnostic strategy to identify persistent infection?

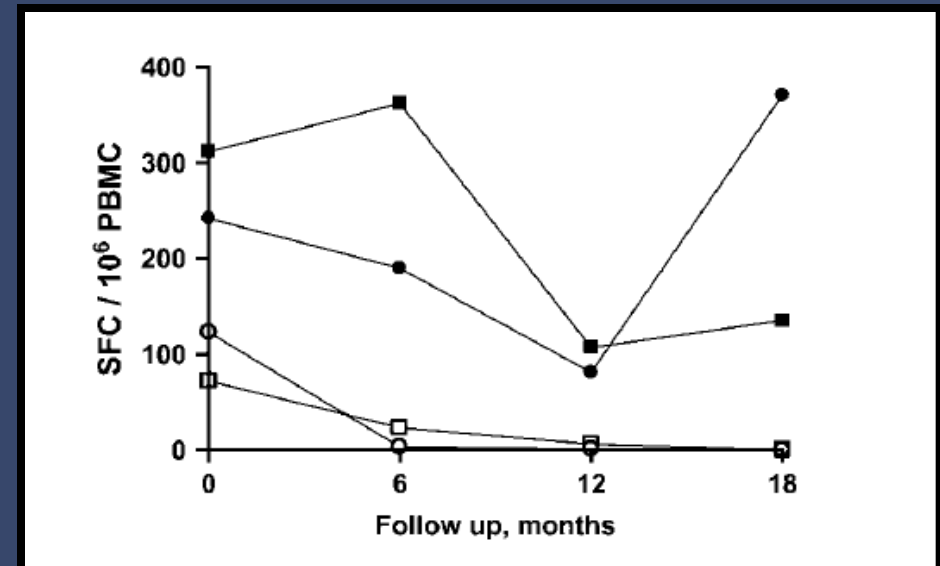
Model of Memory: Possible Outcomes



Transient Exposure?

- Friedman et al., Infect Control Hosp Epidemiol 2006
 - 45 % HCW previously positive by TST were negative on repeat testing
 - Best correlation with persistent TST positivity
 - Foreign birth
 - BCG
 - TST > 15mm
 - Best correlation with TST reversion
 - Working in study clinic

Ewer et al., Am J Respir Crit Care Med
Vol 174. pp 831–839, 2006



Richard L. Riley & William F. Wells



Riley: First Experiment – counted 71 infections

(135 infections if 6 – 13 mm reactions included)

TST reaction	# guinea pigs autopsied	confirmed TB	% confirmed TB
0 – 5 mm (238 guinea pigs)	39	0	0
6 – 13 mm - no necrosis (64 guinea pigs)	24	6	25
6 – 13 mm – necrosis (16 guinea pigs)	16	13	81
14+ mm – necrosis (55 guinea pigs)	55	52	95



The AIR Facility



Data From AIR Facility (Courtesy Ed Nardell)

- 4 months exposure of 360 GPs
- 26 different patients, 15 M, 11 F
 - 60% HIV +
 - Selected for clinical and lab features associated with infectivity (sm+, cavitory, coughing, just started on therapy)
- All infected animals sacrificed, autopsied and cultured at the end of the experiment
 - Infected animals remained in exposure chamber
- Plan: match GP isolates to human source isolates by genotyping
 - 3/14 cultures are XDR
 - 11/20 catalase negative



Transient TB Infection

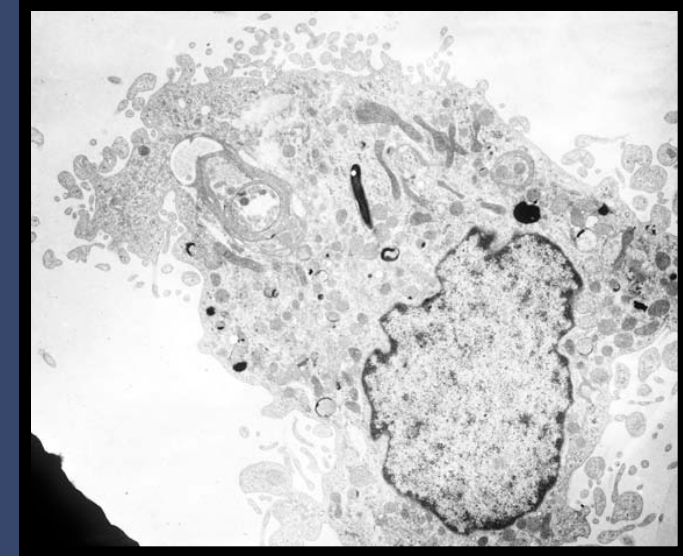
GUINEA PIG TST CATEGORY	# OF GPS	# OF GPS THAT REVERTED	# OF GPS THAT REVERTED AND THEN REACTED AGAIN	% OF REVERTERS
Reacting < 6 mm on TST 2, 3, 4, and 5	91	N/A	N/A	N/A
Max. reaction 6 – 13 mm on TST 2, 3, or 4	163	53	33	32.5
Max. reaction ≥ 14 mm on TST 2, 3, or 4	64	0	0	0
Max. reaction ≥ 14 mm on TST 5	13	N/A	N/A	N/A

Understanding T Cell Memory

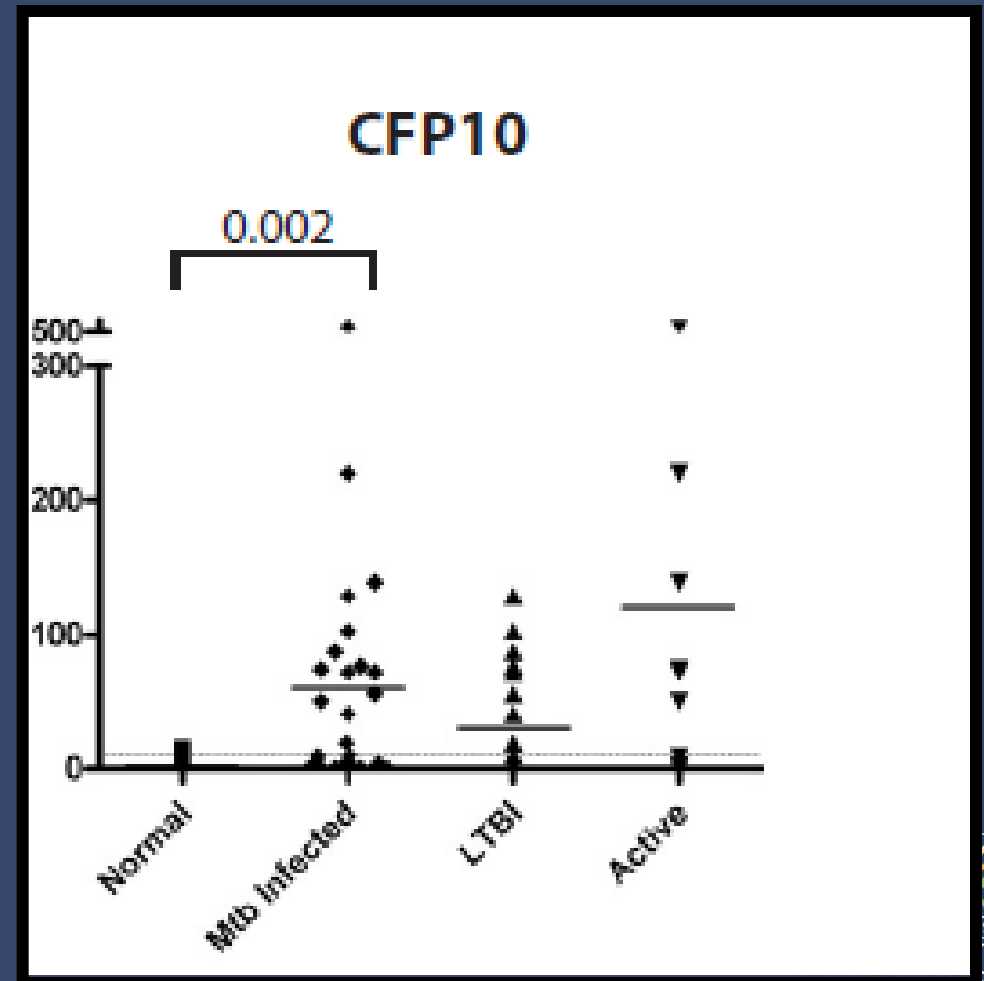
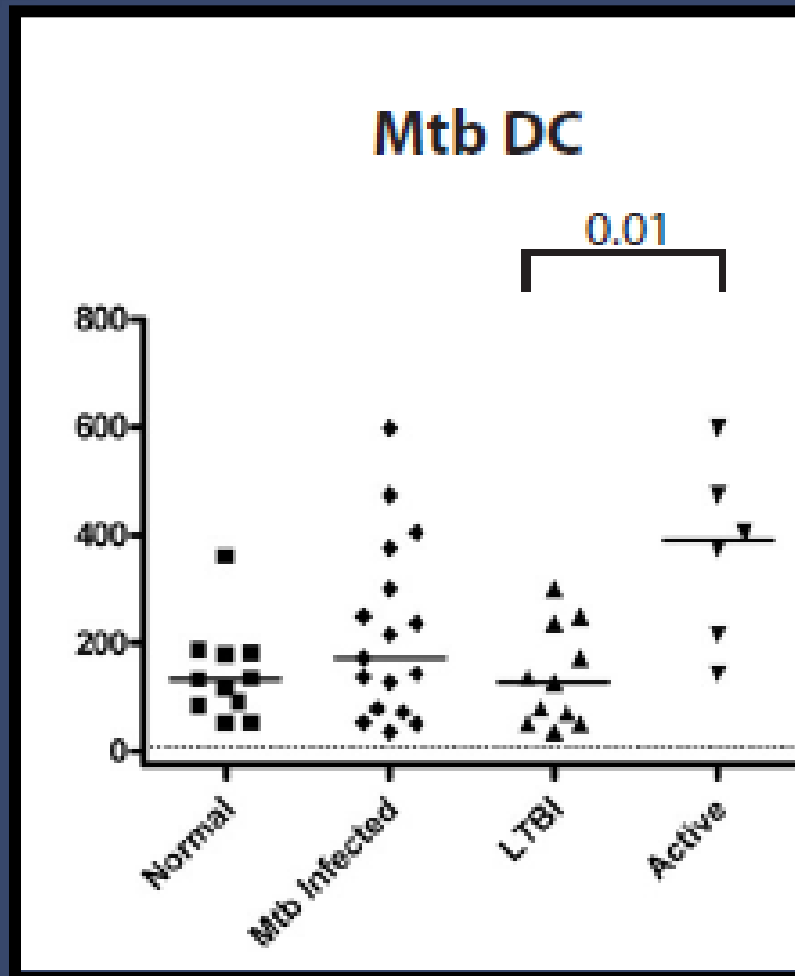
- Can we distinguish exposure from infection?

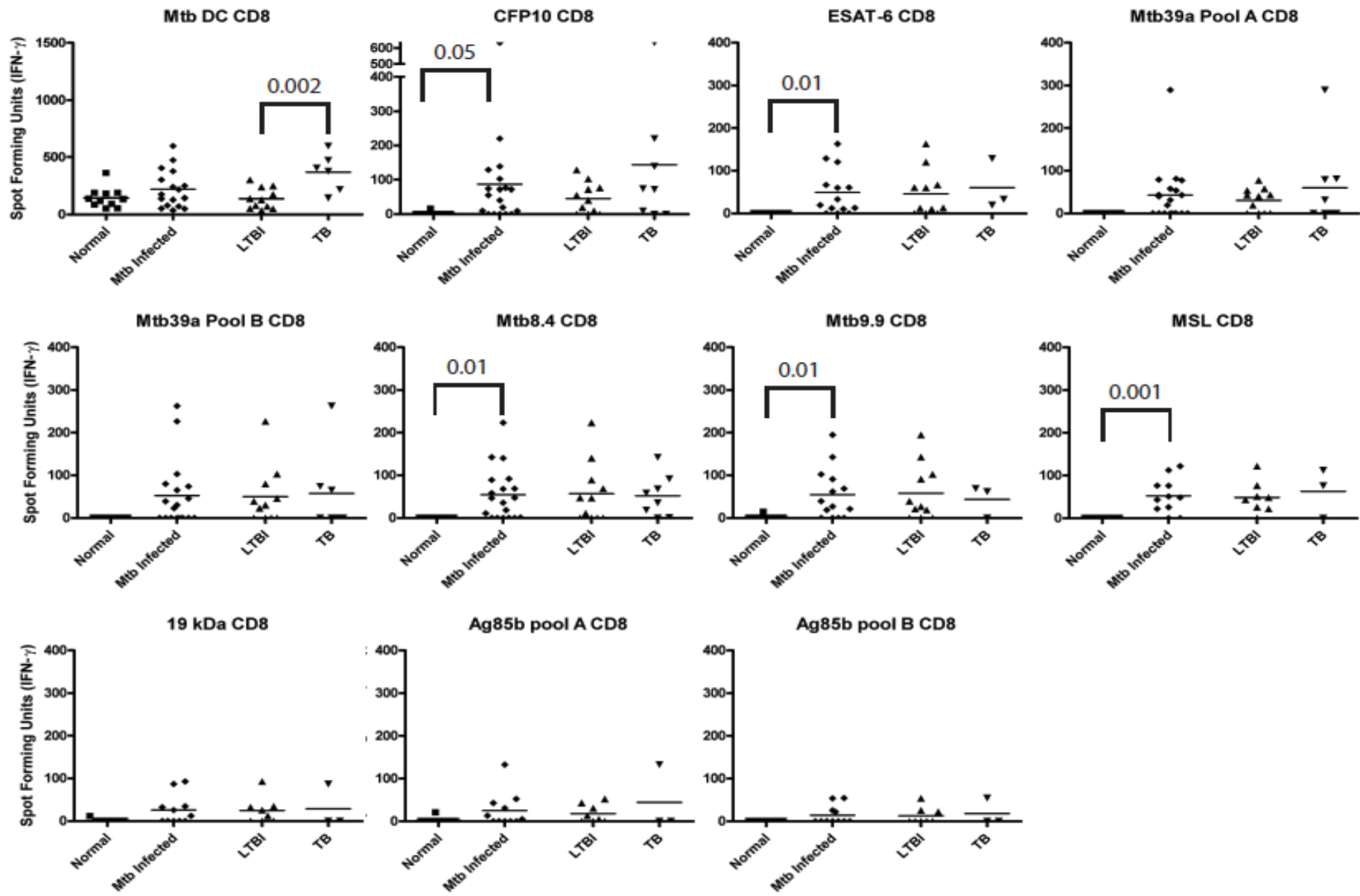
CD8 T Cells

- Present at high frequency in those infected with Mtb (Both active and latent infection)
- Both classically, and non-classically HLA-restricted
- HLA-B dominates the classical response
- Preferentially recognize heavily infected cells, and hence may be a surrogate for bacterial burden and/or disease progression
- Recognize MHCII negative cells
- May play a unique role in chronic/persistent phases of infection

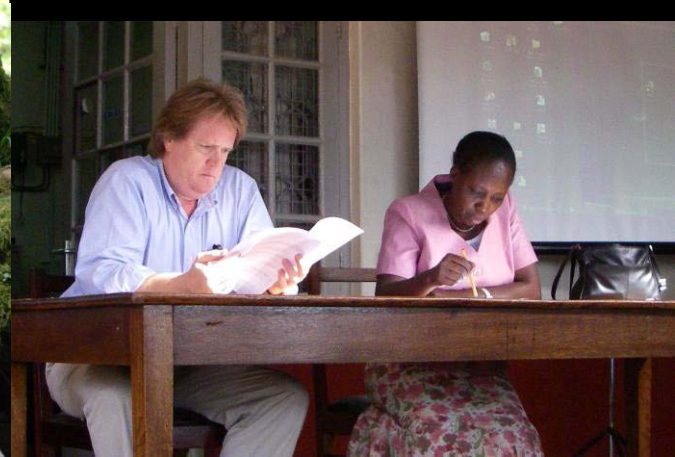


CD8+ T Cells in Human Infection with Mtb





Clinical Evaluation of CD8 Antigens: Uganda CWRU research collaboration - TBRU/Makerere University



Effect of disease and age on T cell immunity

Pediatric TB Disease study



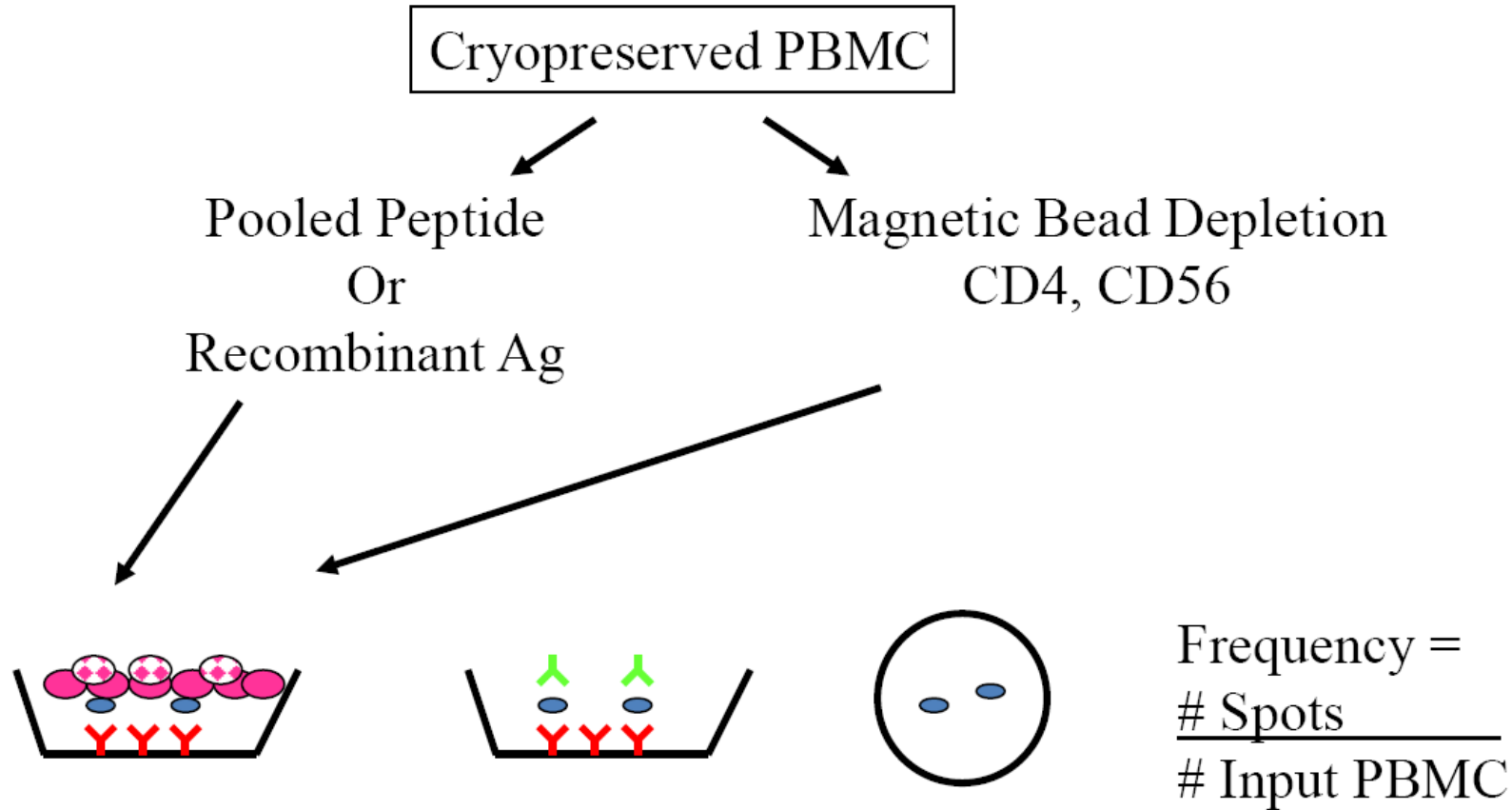
Overall study design of the Pediatric TB disease study (TU study)				
Age groups (years)	< 2 (n)*	2 < 5 (n)*	5 ≤ 10 (n)*	Adults (n)
HIV neg Healthy Exposed	40	30	30	20
HIV neg Localized Disease	40	30	30	20
HIV neg Dissem. Disease	20			
HIV pos Localized Disease	40	30	30	20
HIV neg Dissem. Disease	20			

Pediatric Study: Baseline Characteristics

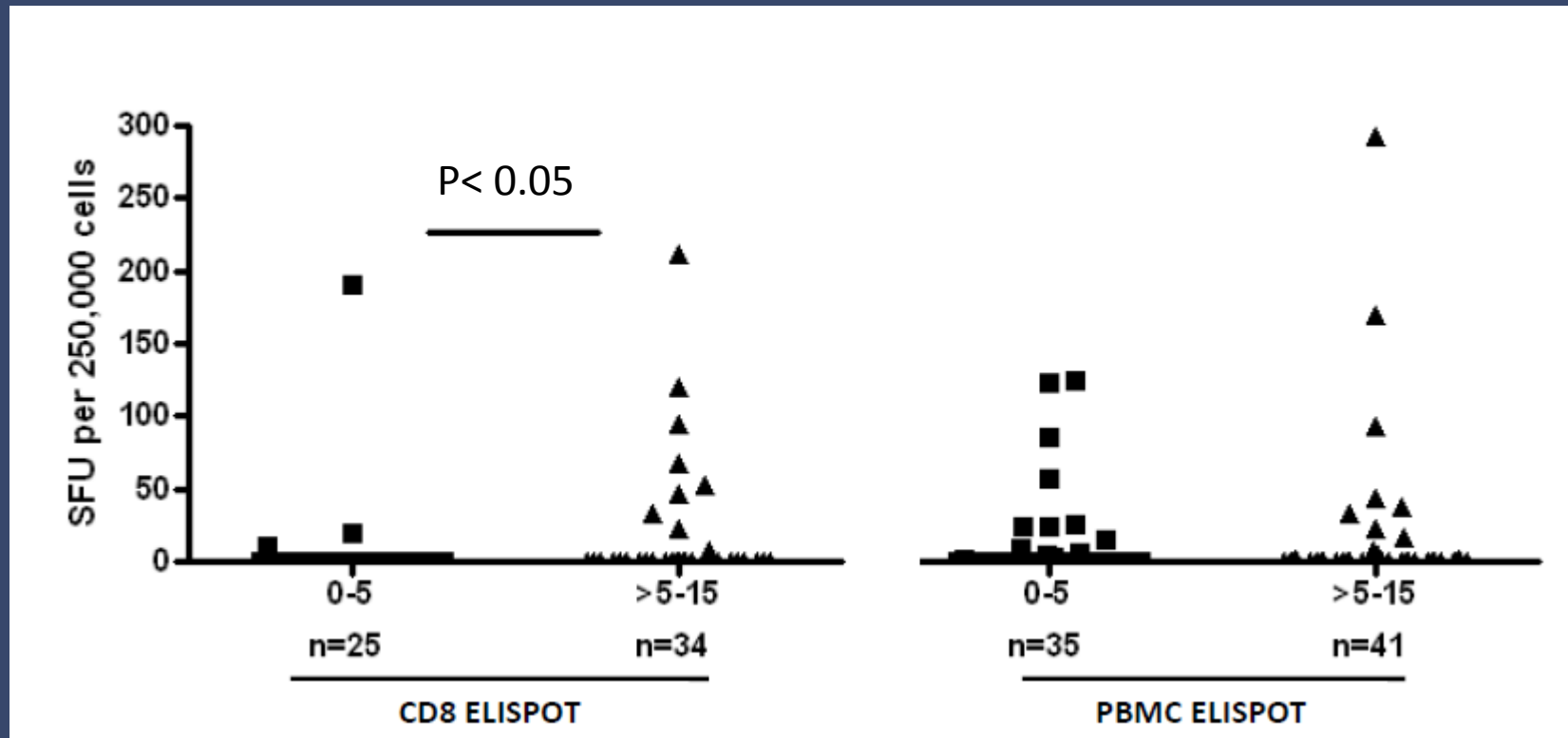
	Healthy Exposed (HE)	Confirmed Probable TB (CP)	p value between HE&CP	Healthy Exposed (HE)	Confirmed TB (Con)	p value between HE & Con	Probable TB	Confirmed TB	p value Between Prob/Con
N	62	96		62	28		68	28	
Median Age (IQR)	4.5 (2.5-6.0)	2.6 (1.0-6.0)	0.02	4.5 (2.5-6.0)	3.0 (1.0-6.5)	0.12	2.5 (1.1 to 6.0)	3.0 (1.0-6.5)	0.8
Female (%)	29 (47)	51 (53)	0.4	29 (47)	11 (39)	0.5	40 (59)	11 (39)	0.08
Median BMI score (IQR)	-0.12 (-0.56 to 0.60)	-0.74 (-2.1 to 0.61)	0.0028	-0.12 (-0.56 to 0.60)	-1.77 (-3.5 to -0.02)	<0.0001	-0.35 (-1.5 to 0.8)	-1.77 (-3.5 to -0.02)	0.005
TST+ (%) 95% CI	28 (45) (0.33 to 0.58)	40 (42) (0.32 to 0.52)	0.6	28 (45) (0.33 to 0.58)	13 (46) (0.27 to 0.66)	0.9	27 (40) (0.28 to 0.52)	13 (46) (0.27 to 0.66)	0.5

ELISPOT Analysis of T-cell Responses in Kwempe, Uganda EX-VIVO

A Positive ELISPOT:
2*STDEV above the Background (media)



Pediatric Study: Household Contacts

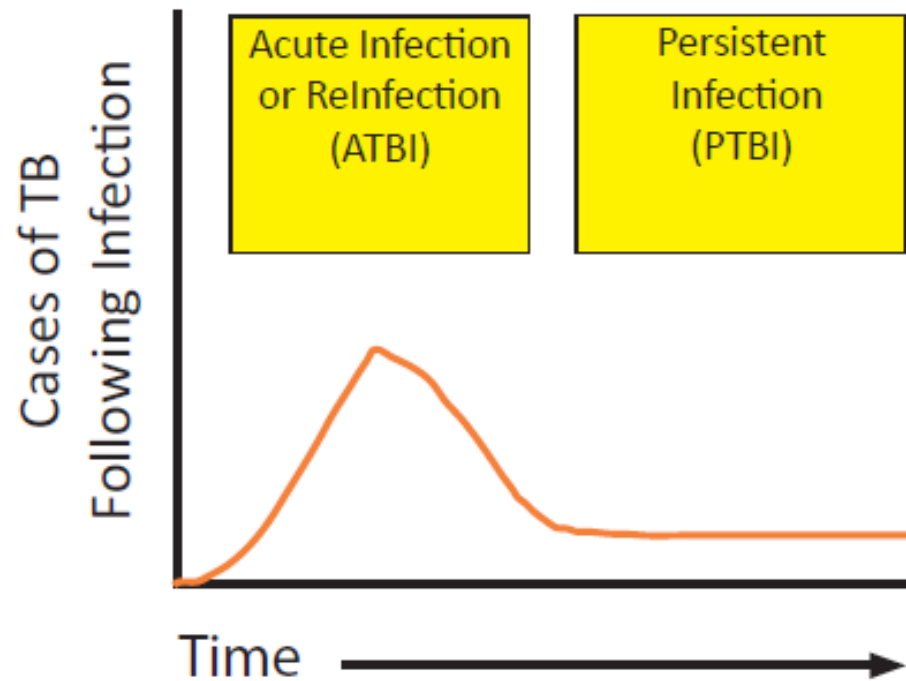


Pediatric Study: Summary

- A positive CD8 is predictive of TB in a young child with pneumonia
- Young household contacts are unlikely to have a CD8 response
 - Exposure vs infection?
 - Reflection of bacteria burden?
 - Prediction:
 - A CD8 response will identify those at risk for progression
- Additional antigens will be needed to improve sensitivity

Rethinking Infection with Mtb

Model of Infection with Mtb



Rethinking the Natural History of Infection with Mtb

Classification of Infection with Mtb (TBI)

	Risk of Progression	Diagnosis			
		TST	CD4	CD8	Biomarker for Acute Infection
Exposure (no infection)	Low	+ (transient)	+ (transient)	-	-
Acute Infection (ATBI)	High	+	+	+	+
Persistent Infection (PTBI)	High in Setting of Immunosuppression	+	Variable	Variable	-

Food for Thought

- How do we better identify those at risk for disease progression?
 - Vaccination trials (Aeras)
 - US Immigration (mandatory TST)
 - Subjects who refuse chemoprophylaxis
 - Treatment trials

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- Collaborators

Dave Sherman
Karen Dobos
John Belisle
Henry Boom
Roy Mugwara
Ole Lund
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