ROLE OF CD8 IN PROGRESSION FROM LATENT TO ACTIVE TB
Disclosures

• David Lewinsohn:
  • OHSU inventor, CD8⁺ T cell vaccines and diagnostics
  • Viti Inc., CEO, current

• Spouse Deborah Lewinsohn:
  • OHSU inventor, CD8⁺ T cell vaccines and diagnostics
  • ViTi Inc., President, current

• OHSU and Drs. Deborah and David Lewinsohn have a financial interest in ViTi, a company that may have a commercial interest in the results of this research. This potential individual and institutional conflict of interest has been reviewed and managed by OHSU.
Using the Host Response to Discern Bacterial Burden

The Big Questions:

• Following exposure to Mtb who is at risk for disease?
• During the course of TB Treatment, can we predict who is likely to relapse?
• Can measuring the host response help us where bacterial burden is low?
Spectrum of Infection with Mtb

Adapted from Young DB et al., Trends Microbiol 2009
Model of Memory: Possible Outcomes

- Active TB
- Latency with Antigenic Persistence
- Transient Exposure to TB

Time
Unique functions of CD8+ T cells

- Recognition of MHC Class II negative cells
- Preferentially recognize heavily infected cells
- Discern bacterial burden
Unique functions of CD8+ T cells

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Lewinsohn DA et al., AJRCCM, 2003
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
CD8+ T cells: A reflection of antigenic load?

- CD8+ T cells assessed in adults with LTBI or TB disease by ICS/FACS (Lausanne, Switzerland)
- CD8+ T cells found more frequently in PTB [67%] than ETB [37%] than LTBI [15%]
- CD8+ T cells at higher frequency in Smear-positive TB than Smear-negative TB

Rozat et al., EJI, 2013
CD8+ T cells as surrogate for bacterial burden in adults

- NAA2/08-0023 CDC / TBRU Collaboration: Kathleen Eisenach & John Johnson
- Longitudinal study in smear positive, HIV negative adults in Kampala, Uganda, initiating treatment
- Blood drawn for ELISPOT analysis
  - Baseline
  - Week 8
  - Week 24

Nyendak M et al, PLoS ONE, 2014
CD8+ T cells as surrogate for bacterial burden in adults

Nyendak M et al, PLoS ONE, 2014
CD8$^+$ T cells as surrogate for bacterial burden in children

- HIV negative Ugandan children <10 yrs hospitalized with pulmonary TB versus healthy household contacts.
- ESAT-6/CFP-10-specific T cells measured by IFN-γ ELISPOT.
- CD8$^+$ T cells (PBMC depleted of CD4$^+$ T cells) and whole PBMC measured.

Lancioni et al, AJRCCM, 2012
CD8\(^+\) T cells as surrogate for bacterial burden in children

Lancioni et al, AJRCCM, 2012
• Define Immunodominant HLA-Ia- and HLA-Ib- restricted Mtb Antigens
  – Lewinsohn Lab(s)
  – Karen Dobos, CSU
  – Dave Sherman, Seattle Biomed
  – William Hildebrand, UOHSC
  – Avigdor Shafferman, IIBR

• Define Clinical Utility
  – Henry Boom, TBRU, CWRU
  – Harriett Myanja & Sarah Kiguli, Makerere University, Kampala, Uganda

• Define Vaccine Utility
  – Helen McShane & Elena Sytlianou, Oxford University
Mtbb Genome
4011 genes
331,000 peptides

Mtbb HN878 genome
(Beijing strain)
3922 ORFs

\~42,000,000 potential 9, 10, 11-mer
binders to 12 HLA supertypes

- Median binding affinity of predicted binders in the
  cluster
- Density of predicted binders in the cluster

CTL Peptide Library
39,000 15-mer peptides
68,713 putative CTL binders

80% representation of the
genome ORFs
\> 95% high affinity epitopes

Genomic Peptide Library
389 Genes
38,989 peptides
T Cell Based Identification of CD8 Antigens

PBMC from Person with Mtb Infection

Antigen

Estimation of Effector Cell Frequencies Ex-Vivo

Clinical Relevance

Mtb-Infected DC

Antigen Identification HLA-Mapping Minimal Epitope

T Cell Clone
Definition of CD8 Antigens Using Pooled Peptides

Subjects
1/2 Pheresis / screen
15 LTBI
5 Active

CD8 T Cells
3x10⁹ PBMC
4.5 x10⁸ CD8

ELISPOT Assay
250,000 CD8 T cells / well
20,000 DC / well
Duplicate wells

Dendritic Cells
3x10⁸ PBMC
3.6 x10⁷ DC

Ex-Vivo Determination of Frequency
• Commonly recognized
• Strongly recognized

Peptide Library
50 peptides / well
5 mcg / peptide
Solid phase synthesis (Jerini)

Controls
Media (7 wells)
PHA
HIV Gag
Ex vivo T Cell Screens of Mtb-infected Donors (target n=20)

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Summary of all ex vivo donor screens (Top 5%)
Clinical screening & validation
Kampala, Uganda

- TBRU Kawempe Community Health Study
  - Identification of commonly recognized antigens
    - 10 Active / 10 LTBI
  - Identification of disease specificity
    - 50 Active / 50 LTBI
Validation Summary
Immunodominant Peptide Pools
Top 5% for Any Donor
At Least Three donors

* Under evaluation per Elena Stylianou and Helen McShane
CD8 antigen combinations

- CD8+ T cell responses in adults with TB disease (Kampala, Uganda)
- HIV positive ($n=20$) & HIV negative ($n=20$)

% of Donors positive by antigen (CD8+ T cells, IFN-gamma)
Role of CD8⁺ T cells in the diagnosis of TB

- Recognition of MHC Class II negative cells
- Preferentially recognize heavily infected cells
- Discern bacterial burden
  - Specific to Mtb-infected individuals.
  - Positive correlation with degree of TB disease
  - Decrease with effective TB treatment
  - Additional sensitivity for TB disease with multiple TB antigens.

Adapted from Young DB et al., Trends Microbiol 2009
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