Recognition and Control of Mtb Infected Cells: From Basics to the Clinic
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Virtual Workshop

The role of cytotoxic lymphocytes in controlling TB

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Presenter Disclosures

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NIH
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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
  - Mucous
  - IgA
  - Surfactant
  - Defensins
  - Cavity

- Mucosa
  - B Cells
  - Mucosal Neutrophils

**Intracellular**

- "Normal" Lung
- Granuloma
- Tuberculoma

- Macrophage

**Defense Mechanisms**

- CD4 T Cells (HLA-II only)
- CD8 T Cells
- HLA-1a
- 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

Table 2: Primary and secondary efficacy endpoints

<table>
<thead>
<tr>
<th>Endpoint Description</th>
<th>Placebo (n=1395)</th>
<th>MVA85A (n=1399)</th>
<th>Vaccine efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endpoint 1 (primary efficacy endpoint)</td>
<td>39 (3%)</td>
<td>32 (2%)</td>
<td>17.3% (13.9 to 28.2)</td>
</tr>
<tr>
<td>Endpoint 2 (exploratory efficacy endpoint)</td>
<td>52 (4%)</td>
<td>55 (4%)</td>
<td>-6.9% (-56.1 to 26.9)</td>
</tr>
<tr>
<td>Endpoint 3 (exploratory efficacy endpoint)</td>
<td>177 (13%)</td>
<td>196 (14%)</td>
<td>-12.1% (-37.4 to 8.5)</td>
</tr>
</tbody>
</table>

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).

Figure 2: Vaccination immunogenecity

(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 21 days after vaccination. (B) Frequencies of cytokine-expressing Ag85A-specific Th1 CD8-positive T cells expressing IFN-γ, TNFα, or interleukin 2 and (C) frequencies of Ag85A-specific Th2/CD4-positive T cells expressing IL-4.
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

Lewinsohn et al., PLoS Pathogens, 2007
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**

- **A** C57BL/6
- **B** Phox⁻/⁻

**Nitric Oxide**

- **A** C57BL/6
- **B** INOS⁻/⁻

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

K. Heran Darwin, Sabine Ehrt, José-Carlos Gutierrez-Ramos, Nadine Weich, Carl F. Nathan

SCIENCE VOL 302 12 DECEMBER 2003
Polycytotoxicity

Granzyme B
Granzyme A
Granulysin
Perforin
IL-26

Busch et al., AJRCCM 2016
Spencer et al., PloS Path 2013
Van Meijgaarden et al., PloS Path 2015
Howerkamp et al. Sci Reports 2020
Live Dead reporter Mtb strain

Sensitive Intracellular Mtb Killing Assay

rAuxotroph Mtb (ΔleuDΔpanCD)

Killing Activity +

Killing Activity -

T cell clone BEAS-2B Dead Mtb Live Mtb

OD600

0.0 0.2 0.4 0.6

0 2 4 6 8 Days

- o - rAxMtb + Zeo
- o - rAxMtb + Leu/Pant + Zeo

Courtesy
Bill Jacobs
Sam Behar
Bryan Bryson

Shogo Soma,
DVM PhD
Differential T Cell Dependent Killing

JE1
(HLA mismatch
CD8 T)

A10
(HLA match
CD8 T)

A3
(Lung MR1-T)

DAPI
mEmerald
RFP(live TB)
Overlay

(60x, 25 fields were stitched)
Non-treat
ABX
D466-A10
D003-JE1
D426-G11
D481-F12
D1004-A3
B1026-A2

(IGA match CD8 T)
(IGA mismatch CD8 T)
(Blood MR1-T)
(Blood MR1-T)
(Lung MR1-T)
(Lung MR1-T)

0.4267
0.3874
0.0010
<0.0001
<0.0001
<0.0001

0.1105

Live Mtb (RFP pixel)/# of BEAS-2B

WT-BEAS2B
MR1KO-BEAS2B

0 200 400 600 800
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