Preclinical profiling of lung granulomas to identify potential correlates or mechanisms of protection

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Critical host-pathogen interactions occur in the complex microenvironment of a granuloma.
**TOOLS AND TECHNOLOGIES FOR MACAQUE TB STUDIES**

**Barcoded Mtb Erdman**
A unique DNA barcode for each bacillus*

**Serial PET CT imaging**

**Detailed necropsies**
Obtain all scan-matched lesions and lymph nodes

**Serial blood and BAL**

- Quantitative microbiology (CFU, CEQ, barcode)
- PET CT history for each lesion (SUV, size, space, timing)
- Immunohistochemistry on lesions
- 25+ color spectral flow cytometry on lesions, lung, LNs
- Samples for scRNAseq, Systems Serology, innate assays

*Martin, Cadena et al mBio 2017
Some granulomas restrict Mtb but some do not

Each symbol is a granuloma, each column shows the granulomas from a single monkey

Each granuloma arises from a single bacterium
(Lin, Ford et al Nat Med 2014; Martin, Cadena et al mBio 2017)

Pauline Maiello and in Gideon, Hughes, Tzounas et al Immunity 2022
How to determine successful or detrimental host functions in granulomas?

Resolving granuloma (killing Mtb)

Permissive granuloma (Mtb growth, dissemination)
Single cell RNAseq using SeqWell to define features of restrictive and permissive granulomas

4 Cynomolgus Macaques
Low-dose Mtb Infection
Serial PET-CT

10 weeks p.i.
Granuloma Isolation
Dissociation

Granuloma-level Assays
CFU Assays
Mtb Chromosomal Sequencing (CEQ)

26 randomly chosen granulomas
Single-cell Sequencing ~15000 cells/array
Single-cell Analysis

All in BSL3
Gideon, Hughes, Tzounas, et al Immunity, 2022
Range of bacterial burden in the randomly selected granulomas for scRNAseq

Granulomas for scRNAseq

26 granulomas

Granulomas

CFU

CEQ*

CFU/CEQ*

Animal number

3817 3917 4017 4217

High

Low

High

Low

High

Low

p<0.0001

p=0.07

p=0.03

Cell types within the granuloma that correlate positively or negatively with bacterial burden

109,584 cells from 26 granulomas

- **B cells**
  - Rho: 0.15 p=0.5

- **Plasma Cells**
  - Rho: 0.73 p<0.0001

- **T/NK cells**
  - Rho: -53 p=0.005

- **Macrophages**
  - Rho: 0.02 p=0.09

- **Neutrophils**
  - Rho: 0.04 p=0.8

- **Mast cells**
  - Rho: 0.57 p=0.002

- **cDCs**
  - Rho: 0.28 p=0.2

- **pDCs**
  - Rho: 0.07 p=0.7

- **Endothelial cells**
  - Rho: 0.6 p=0.001

- **Fibroblasts**
  - Rho: 0.49 p=0.01

- **T1P**
  - Rho: 0.51 p=0.008

- **TP2**
  - Rho: 0.2 p=0.3
Negative correlation with bacterial burden
T1/T17 is composed of CD4 and CD8 T cells (Th1* or exTH17?)

The cytotoxic CD8 subpopulation but not the CD4/CD8 IFNγ/TNF subpopulation correlates with reduced bacterial burden.
Correlated abundance of cell subsets in granulomas

1. Type 2
2. T1/T17 & cytotoxic

Group Proportion

CFU

Granuloma

High

Low

Burden

Animal

Pearson Correlation Across Granuloma Compositions

-0.5 0 1
High bacterial burden (early) granulomas are characterized by a reinforcing Type 2 response

Gideon, Hughes, Tzounas, et al Immunity, 2022
Most granulomas with low bacterial burden (and better killing) are late forming...most likely in the context of an adaptive immune response.
Evolution of T cell responses in granulomas

- **Early**
  - N=2
  - Chinese cynomolgus macaques (CCM)
  - 4 weeks: Identify original granulomas by PET CT
  - Necropsy at 4 weeks p.i.

- **Mid**
  - N=3
  - Develop for 8 weeks
  - Necropsy at 12 weeks p.i.

- **Late**
  - N=3
  - Develop for 16 weeks
  - Necropsy at 20 weeks p.i.
Transcription factor expression in lymphocytes increases in mid and late timepoints

Each point represents an individual granuloma, colored by animal.

Grant et al Cell Reports 2022
Adaptive lymphocytes have increased T-bet expression at mid and late timepoints.

Each point represents an individual granuloma, colored by animal.

Grant et al Cell Reports 2022
Frequency of T-bet$^+$ cells in granulomas negatively correlates with bacterial burden

All lymphocytes (except B cells)

Spearman $r=-0.4715$  
$p=<0.0001$

CD4+ T cells

Spearman $r=-0.3358$  
$p=0.0023$

CD8+ T cells

Spearman $r=-0.4486$  
$p=<0.0001$

Each point represents an individual granuloma, colored by animal
Identifying *Mtb*-specific CD4$^+$ T cells in NHPs

Mauritian Cynomolgus Macaque (MCM) model

- Isolated for >500 years
- Limited MHC diversity

4 tetrarmers used to identify Mtb-specific T cells

- DPA/DPB Rv1196$^{371-385}$
- DPA/DPB Rv0125$^{81-92}$
- DRA/DRB CFP-10$^{36-48}$
- DRA/DRB CFP-10$^{71-85}$

Tetrarmers synthesized by NIH tetrarm core

CFP10 previously mapped by O’Connor et al.

Grant et al mBio 2023
Mtb-specific cells in the lungs and granulomas

Each point represents an individual sample, colored by animal.

Grant et al mBio 2023
Frequency of Mtb-specific cells is positively correlated with CFU, but functionality is negatively correlated.
Intravenous BCG provides superior protection against infection and disease

### Rhesus macaques

<table>
<thead>
<tr>
<th>Group</th>
<th>BCG Dose</th>
<th>N group</th>
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</thead>
<tbody>
<tr>
<td>Naive</td>
<td>N/A</td>
<td>4</td>
</tr>
<tr>
<td>ID&lt;sub&gt;Low&lt;/sub&gt;</td>
<td>$5 \times 10^5$</td>
<td>10</td>
</tr>
<tr>
<td>ID&lt;sub&gt;High&lt;/sub&gt;</td>
<td>$5 \times 10^7$</td>
<td>8</td>
</tr>
<tr>
<td>Aerosol (AE)</td>
<td>$5 \times 10^7$</td>
<td>10</td>
</tr>
<tr>
<td>ID/AE</td>
<td>$5 \times 10^5$ / $5 \times 10^7$</td>
<td>10</td>
</tr>
<tr>
<td>Intravenous (IV)</td>
<td>$5 \times 10^7$</td>
<td>10</td>
</tr>
</tbody>
</table>

12 weeks post-infection

Total thoracic bacterial burden

![Image of bacterial burden](image_url)
BCG IV induces T-resident memory cells in lungs

4 weeks post-BCG
CD45IV staining
CD45IV Ab delivered just prior to necropsy of the macaques
CD45IV negative cells are in tissues not blood
Resident cytotoxic CD8 T cells are increased in lungs of BCG IV vaccinated macaques

CD4 T cells
- Unvaccinated: 0.84, 1.01
- BCG IV: 0.60, 2.37

CD8αβ T cells
- Unvaccinated: 35.8, 27.8
- BCG IV: 17.9, 9.35

Granulysin
- Unvaccinated: 17.9, 9.35
- BCG IV: 18.2, 19.8

Granzyme B
- Unvaccinated: 51.3
- BCG IV: 41.5

Perforin
- Unvaccinated: 10.1
- BCG IV: 21.5

Andrew Simonson

Uninvolved lung at necropsy
Summary

• Several T cell phenotypes in granulomas correlate with control of Mtb
• CD4 and CD8 T cell phenotype/function correlate with control of infection
• Transcription factor expression in Mtb specific T cells correlate with control of infection
• IV BCG induces strong T-resident memory cell populations in lungs and leads to rapid elimination of Mtb
• Ongoing Mtb infection (active or LTBI) can protect against reinfection (concomitant immunity)
• Broad and durable T cell responses to multiple antigens may be needed for protection against infection
• Consider strategies that increase CD4 and CD8 T resident memory cells, including CD8s making cytotoxic effectors
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