Learning from the NHP TB model – Immunology of “pre-granulomatous” infection

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Dr. Picker is a founder of, and consultant for, Vir Biotechnology, Inc. OHSU has licensed CMV vector technology to Vir, a company in which both OHSU and Dr. Picker have significant financial interest. Potential individual and institutional conflicts of interest have been reviewed and managed by OSHU.
Rhesus macaque (RM) TB pathology closely resembles that of humans, but . . .

. . . disease progression in RM is more frequent and faster than in people . . .

(making this a very stringent model for vaccine testing)
Indeed, TB in RMs is quite resistant to conventional vaccination, including intradermal BCG:

But remarkably, this has not been the case for some novel vaccine approaches . . .
Two different vaccines – RhCMV/TB and IV BCG – have shows early complete control* of Erdman stain *Mtb* in Indian-origin rhesus macaques:

*prevention of detectable (granulomatous) disease

Prevention of tuberculosis in rhesus macaques by a cytomegalovirus-based vaccine

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These findings have prompted our group to explore the early immunology of \textit{Mtb} infection in RMs to identity responses mediate TB control prior to development of overt granulomas . . .

Program Goals (Cascade ImpacTB consortium):

1. Characterize immune landscapes in the RM lung and lung-draining lymph nodes associated with the capacity to eradicate or stringently control a subsequent \textit{Mtb} challenge.

2. Define early immune events in the lung and lung-draining lymph nodes that lead to \textit{Mtb} eradication or stringent containment prior to overt granulomatous disease.
Experimental Approach: Serial necropsy study with focus on “pre-granuloma” immunity

RM cohorts (n=4-8 RM per timepoint per group)
- Unvaccinated
- Intradermal BCG (birth)
- Intradermal BCG (adolescence)
- RhCMV/TB (adolescence)
- IV BCG (adolescence)

Sample collection/distribution

Study Protocol

(2 Erdman Mtb* bact. I.B.)

*BEI Barcoded Erdman Mtb NR-50781)

(Mock challenge)

Control necropsy cohorts

Assays
1. High Dimensional Flow Cytometry (Hansen)
   - Phenotyping
   - ICS
2. Advanced Imaging & Spatial Transcriptomics (Estes)
3. Single cell “Cite-Seq” (Bimber)
4. Bulk RNA-Seq (Gale)
5. Mtb culture/PCR (Hansen/Lifson)
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Sample collection/distribution

Serial necropsies

Study Protocol

| Week | Blood | CT Scan
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Serial necropsies

Low dose challenge

Today's talk will focus on the first 47 necropsies (unvaccinated, BCG vaccinated, and controls)
All day 28 *Mtb* challenged RM show disease, indicating effectiveness of “2 bug” challenge.  
All gross disease contained in challenged (right) lung and draining LNs of challenged lung.  
Extent of disease was not reduced by BCG vaccination; indeed, BCG vaccinated RM appeared to promote both earlier disease onset and more lesions.
Development of *Mtb*-specific T cell responses in lung by ICS:

- *Mtb*-specific T cell responses don’t accumulate in lung until day 21 in unvaccinated RM and are maximal at day 28.
- In BCG vaccinated RM, low level *Mtb*-specific T cell responses are present at the earliest examined (day 14) timepoint and increase (albeit modestly) over the following 2 weeks.
- Day 28 responses are comparable between unvaccinated RM and the majority of BCG vaccinated RM.

Scott Hansen
Development of TB disease (day 28; unvaccinated)

Necrotizing Granuloma

CD4+ T cells / Macrophage
PMNs (MPO)
Mtb Erdman RNAscope
Development of TB disease (day 21; unvaccinated)

Day 7  Day 14  Day 21  Day 28

Intermediate Granuloma

CD4+ T cells / Macrophage  PMNs (MPO)  Mtb Erdman RNAscope
Development of TB disease (day 14; unvaccinated)

Day 7 Day 14 Day 21 Day 28

Nascent Granuloma

CD4+ T cells / Macrophage PMNs (MPO) Mtb Erdman RNAscope
Development of TB disease (day 7; unvaccinated)

Proto-Granuloma

CD4+ T cells / Macrophage

PMNs (MPO)

Mtb Erdman RNAscope
Development of TB disease (unvaccinated vs. ID BCG vaccinated)

Unvaccinated RMs (Lung)

BCG at Birth (Lung)

BCG as Adolescents (Lung)

Average Size of Granuloma (µm²)

D7 D14 D21 D28

Average Size of Granuloma (µm²)

D7 D14 D21 D28

Average Size of Granuloma (µm²)

D7 D14 D21 D28

5mm

5mm

5mm
Multi- and hi-plex imaging approaches delineate immune cell composition and inflammatory landscape during early granuloma development

Starting with mature (day 28) granulomas:

IDO1 = Indoleamine 2, 3-dioxygenase 1

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1mm

1mm

1mm
This high-dimensional immune profiling revealed that IDO-1 is a feature of the earliest recognizable proto-granulomas.
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In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of *Mycobacterium tuberculosis*.

Thus, this mediator of immunosuppressive tryptophan catabolism is present very early in granuloma development!
Transcriptomic discovery tools for granuloma development

**Spatial Transcriptomics:** nanoString GeoMx Digital Spatial Profiler (DSP)

- Regional, but not single-cell, whole transcriptomic analysis, but with spatial context

**CITE-seq:** Cellular Indexing of Transcriptomes and Epitopes by Sequencing

- High resolution single cell whole transcriptomic and cellular phenotypic analysis, but without spatial context

Jake Estes

Ben Bimber
Spatial Transcriptomic Analysis (DSP) of Granuloma Development

Unsupervised Clustering of ROIs

Gradient of Expression by Granuloma Stage
Integration of Spatial and scRNA-seq to Better Understand Granuloma Development

**CITE-seq Resolves Cell Type/Lineage**

GeoMx DSP late granuloma signature

**Signature Maps to Subset of M1-like Macrophages + mDCs**

Use molecular signature to track/quantify cells across samples

L/R caudal lung, n=147,418 cells

**IDO1 Macrophages DAPI**

Expand molecular signatures and identify novel markers at single cell level

D7 Unvaccinated

**PLAAT4**

(retinoid-induced)

Phospholipase A/Acyltransferase 4
CITE-Seq reveals limited/late T cell activation and clonotypic skewing in lung

CITE-seq analysis showing unsupervised clustering of caudal lung T/NK cells (all)

Relatively little cytokine expression

Activation Score Gene Set: TNFSF14, NFKBID, CCL3, CCL4L1, IRF8, IFNG, PLEK, RGCC, NR4A2, NR4A3, LOC100426537, LOC114673087, KLF10, GADD45B

Fraction Activated* Right caudal lung

T Cell Proliferation and Clonal Expansion

TCR Clonotypic Hierarchies, Day 28

T and NK cell activation
Conclusions

1. Findings indicate a clear developmental sequence of granuloma formation:
   • Initial loose associations of macrophages and T cells that predate evidence of adaptive responses in unvaccinated RM,
   • progressive accumulation and tighter association of macrophages with PMN influx, increasing T cells (including *Mtb*-specific), and finally
   • necrosis

2. ID BCG vaccination enhances/accelerates this sequence of events

3. Single cell and spatial transcriptomic analysis reveals:
   • Early induction of immunosuppressive pathways (IDO1)
   • New markers of granuloma myeloid cell differentiation (PLAAT4)
   • Limited/late T cell activation

4. Future analysis will reveal the ability of RhCMV/TB and/or IV BCG vaccine-induced immunity to disrupt this sequence of granuloma development by early control of infection
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