Insights into features of bacterial control from single cell profiling of TB granulomas

Hannah Priyadarshini Gideon, PhD
Department of Microbiology and Molecular Genetics and Center for Vaccine Research
University of Pittsburgh School of Medicine
hpg2@pitt.edu

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On Behalf Of …

Travis Hughes  
(Shalek Lab)

JoAnne Flynn

Alex Shalek

Sarah Fortune
Mtb infection results in granuloma formation

Immune microenvironment

Bacterial growth, persistence and killing

Dynamic Independent

NHP granuloma

Lymphocytes

macrophages

Caseum

NHP granuloma
Heterogeneity in granuloma bacterial burden

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

Granuloma heterogeneity

• One bad apple...
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
4, 8 & 10 weeks p.i.

Granuloma Isolation
Dissociation

Granuloma-level Assays
Colony Forming Units (CFU)
Mtbp Chromosomal Sequencing (CEQ)

Single-cell RNA Sequencing
Single-cell Analysis

Bacterial Burden (CFU - Live)

26 granulomas from 4 animals

Our randomly chosen granulomas were a mix of original/early (seen at 4 weeks by scan) and late (seen at 10 weeks).

Dissemination leads to formation of new granulomas.

Also possible that there are more slowly growing granulomas.

Granuloma size measured in PET-CT scan

15/26: Early granuloma
11/26: Late granuloma

Late granulomas have lower bacterial burden and higher killing than late granulomas

Bacterial Burden
CFU (Live)

CEQ (live + dead)

CFU/CEQ (killing)

15/26: Early granuloma
11/26: Late granuloma

The TB granuloma landscape

26 granulomas

109,584 assigned high quality granuloma cells

13 canonical cell types
Granuloma cellular composition differs by time of appearance and bacterial burden.

Early Granuloma
- T/NK cells: 30%
- Macrophages: 20%
- B cells: 10%
- Neutrophil: 5%
- T2P: 5%
- Mast cells: 5%
- pDC: 5%
- RBC: 5%
- cDC: 5%
- Fibroblasts: 5%
- Endothelial cells: 5%
- Plasma cells: 5%
- T1P: 5%

Late Granuloma
- T/NK cells: 50%
- Macrophages: 20%
- B cells: 10%
- Neutrophil: 5%
- T2P: 5%
- Mast cells: 5%
- pDC: 5%
- RBC: 5%
- cDC: 5%
- Fibroblasts: 5%
- Endothelial cells: 5%
- Plasma cells: 5%
- T1P: 5%

High CFU
- T/NK cells: 20%
- Macrophages: 40%
- B cells: 10%
- Neutrophil: 5%
- T2P: 5%
- Mast cells: 5%
- pDC: 5%
- RBC: 5%
- cDC: 5%
- Fibroblasts: 5%
- Endothelial cells: 5%
- Plasma cells: 5%
- T1P: 5%

Low CFU
- T/NK cells: 40%
- Macrophages: 20%
- B cells: 10%
- Neutrophil: 5%
- T2P: 5%
- Mast cells: 5%
- pDC: 5%
- RBC: 5%
- cDC: 5%
- Fibroblasts: 5%
- Endothelial cells: 5%
- Plasma cells: 5%
- T1P: 5%

More T/NK cells in late (low CFU) granulomas
More mast cells and plasma cells in early (high CFU) granulomas

T and NK cells as mediators of bacterial control

13 T/NK subclusters

41,222 T/NK cells

Subclusters are not strictly CD4 or CD8
Function overrides cell type

Diversity of Cytotoxic cells in granuloma

Granzymes, Granulysin, Perforin, CD8A, CD8B, Conventional CD8T cells, NK, NKT, gd Tcells
T1-T17, Cytotoxic and Stem-like clusters associated with late granuloma and bacterial control

Cytotoxic C4
CD8αβ, PRF1, GZMB, GZMH, GZMM, CXCR1, S100A10

Cytotoxic 5
CD8αβ, GZMK

Stem-like
PLK2, TCF7, CCR7, IL7R, TXNIP, CD69, and ITGB1

Treg
FOXP3, CTLA4, CGA, TIGIT, GATA3, TNFRSF18

T1-T17

T1/T17 hybrid cluster of CD4 and CD8 T cells is strongly associated with control of Mtb

SubPopulation 1
CD4, IL7R, TXNIP

SubPopulation 2
CD8AB, IL7R, cytotoxic effectors

SubPopulation 3
CD8AB, IFNG, TNF

Cellular ecology of TB lung granuloma

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Pearson Correlation Values

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Distinct interaction patterns between early and late granulomas

Early granuloma: canonical Type 2 (IL-13) cytokine signaling
Loss of Mtb control

Late granuloma: T1/T17, Stemlike and Cytotoxic

Cell-Cell interactions Receptor-Ligand

Mast cell
Plasma cell
Macrophages
T/NK cells

**Summary**

Early Granuloma
High Bacterial burden

Late Granuloma
Low Bacterial burden
(Mtb killing)

Mast cells
Plasma cells
B cells
Endothelial cells

T/NK subtypes
T1T17 cells
Stem-like cells
Cytotoxic cells

Primed immune environment

Acknowledgments...

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