Growth inhibition assays as correlate of protection

Stop TB Partnership Working Group on New TB Vaccines (WGNV) and the National Institute for Allergy and Infectious Diseases (NIAID) workshop, June 14, 2023

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Phagocytosing cells: monocytes/ Mf/ DC/ granulocytes

T-cell  B-cell  Antibodies  NK-cell

Mtb infection – Immune responses

Can Mtb be eliminated?

frequency  phenotype  function
Functional assessment of mycobacterial growth control

Unbiased approaches: Analyse immune system as a whole rather than isolated components

- Functional assays to assess the capacity to eliminate mycobacteria > **Mycobacterial Growth Inhibition Assay (MGIA)**

**Optimal performance:**
- Standardized and validated batch of bacteria
- Rapid handling of PBMCs
- Duplicate samples for testing

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Hoft et al, J Infect Dis. 2002;186(10):1448-57
Tanner et al, Vaccine. 2016;34(39):4656-4665
Brennan et al, Clin Vaccine Immunol. 2017; 24(9)
Tanner et al, J Immunol Meth. 2019; 469:1-10
Strong BCG growth control upon recent exposure

Highest control of BCG outgrowth observed in recently exposed individuals, unrelated to IGRA

Joosten, J Clin Invest, 2018
T-SNE identifies CD14dim and CXCL10 in growth control

Joosten, J Clin Invest, 2018
Non classical monocytes produce CXCL10 that mediates control

CD14dim monocyte: Non classical monocytes
Typically express CD16 (FcyRIII) > important in phagocytosis, associated with anti-viral response

Joosten, J Clin Invest, 2018
300BCG study Nijmegen:

- Primary BCG vaccination in healthy adults
- Samples collected prior to vaccination and 3 months post BCG vaccination
- BCG Bulgaria, intradermally

42 participants selected to contain 21 good and 21 poor responders to *Staphylococcus aureus* (SA) stimulation with subsequent IL-1β production as marker of trained immunity.

- MGIA
- scRNAseq unstimulated/ LPS stimulation
MGIA control may exist already before BCG vaccination

**MGIA control definition:**

- **control:**
  - Pre vacc logCFU < 1.38 (= -1log mean inoculum) or
  - \( \Delta \text{logCFU}_{V3,V1} < -0.17 \) (SD mean inoculum)

- **no control:**
  - \( \Delta \text{logCFU}_{V3,V1} > -0.17 \)

Van Meijgaarden, Li et al, in revision
Differential gene expression in relation to control

Van Meijgaarden, Li et al, in revision
Already vs acquired samples are different

- Already controlling vs. acquired control
- Pre-vaccination vs. post-vaccination

Statistical analysis: Increased DEGs in BCG post-vaccination compared to pre-vaccination.

Van Meijgaard, Li et al, in revision
Summary – already vs acquired control

pre-vaccination

already controlling: ↑ type I IFN response
↑ metabolic activity: ox phosphorylation, fatty acid oxidation

post-vaccination

acquired control: ↑ metabolic activity: glycolysis
↑ antigen processing/presentation
↑ T-cell responses
↑ migration

already controlling vs acquired control

↑ response to virus
↑ metabolic activity
↑ type I IFN response

↑ leucocyte activation
↑ cell killing
↑ response to chemokine
↑ response to bacteria

Van Meijgaarden, Li et al, in revision
Growth inhibition assays and protection

IV BCG protects against MTb challenge
(Darrah et al, Nature 2020)

In collaboration with Tricia Darrah, Mario Roederer, Bob Seder
Functional measurement of effector responses is important to evaluate protective immunity,

But may also provide novel insights in host defense mechanisms: monocytes are important, but require interaction with T-cells

Natural control of BCG outgrowth involves different players as control induced upon BCG vaccination

_in vivo_ protection is reflected by increased growth inhibition _in vitro_

Mechanism and persistence of natural control need further investigation
Acknowledgements

LUMC, Dept of Infectious Diseases, Leiden, The Netherlands
Krista E van Meijgaarden
Sandra M Arend
Corine Prins
Paula Niewold
Marjolein van Wolswinkel
Linda Voogd
Amy de Waal
Simone A Joosten
Tom HM Ottenhoff

Norwegian Institute of Public Health, Oslo, Norway
Fredrik Oftung
Gro Ellen Korsvold

KNCV Dutch Tuberculosis Foundation, The Hague, The Netherlands
Sandra Kik

INMI, Rome, Italy
Delia Goletti

Radboud UMC, Nijmegen, The Netherlands
Reinout van Crevel
Rob Arts
Simone Moorlag
Valerie Koeken
Mihai Netea

Ragon Institute, Boston, USA
Patricia Grace
Galit Alter

Helmholtz Centre for Infection Research, Hannover, Germany
Wenchao Li
Yang Li

VRC, NIAID, NIH, USA
Patricia Darrah
Mario Roederer
Bob Seder

Infectious Disease Unit, St. John’s Research Institute, Bangalore, India
Annapurna Vyakarnam