



Leiden University  
Medical Center

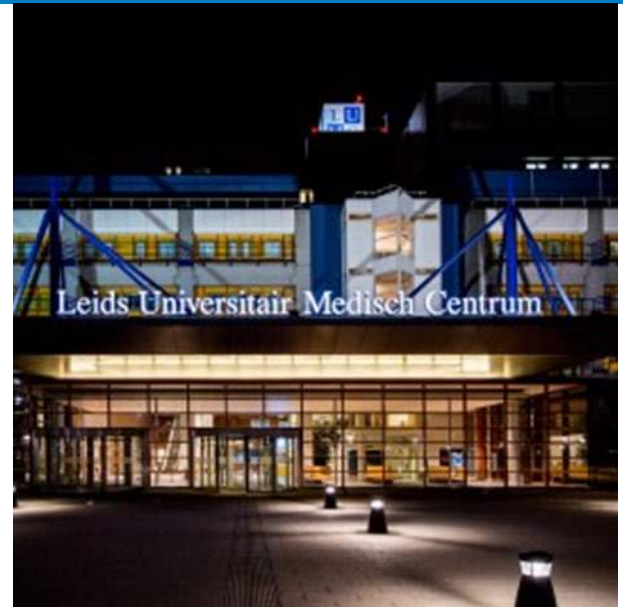
# Growth inhibition assays as correlate of protection

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

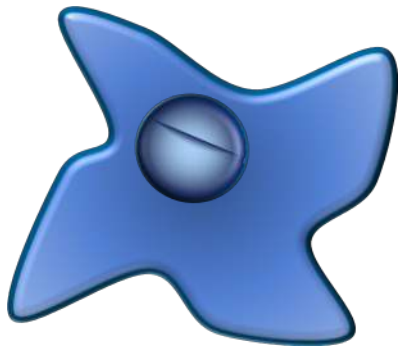
Simone A Joosten

Infectious Diseases

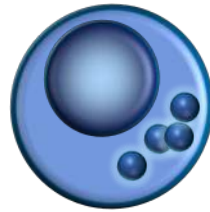
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# Mtb infection – Immune responses



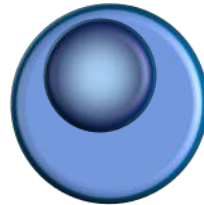
Phagocytosing cells:  
monocytes/ Mf/  
DC/ granulocytes



T-cell



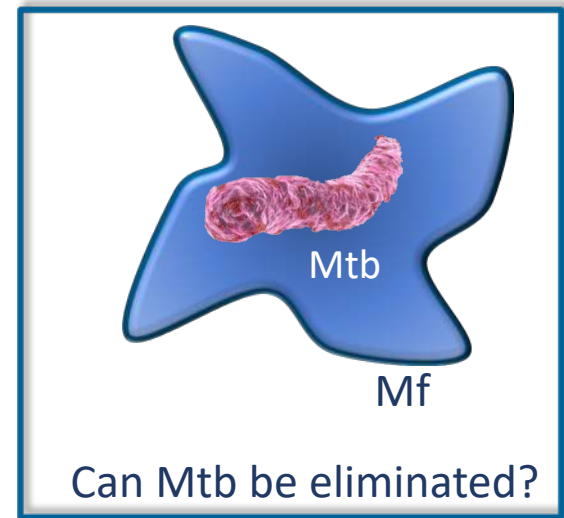
NK-cell



B-cell



Antibodies



Can Mtb be eliminated?

frequency

phenotype

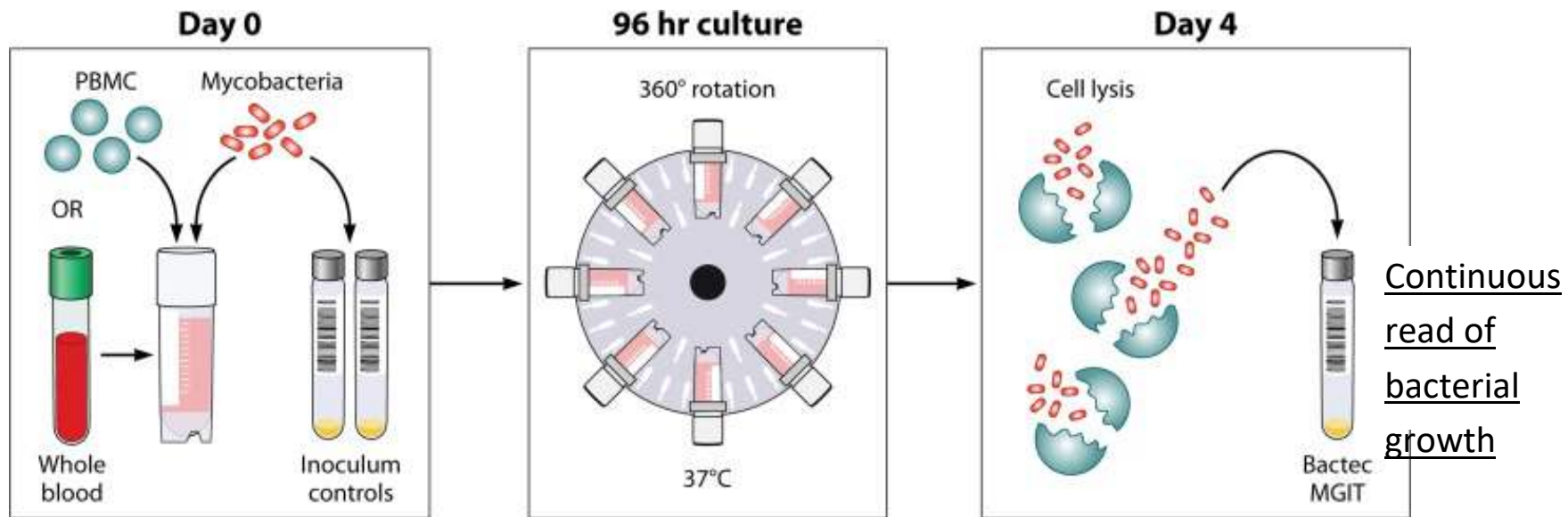
function

# 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

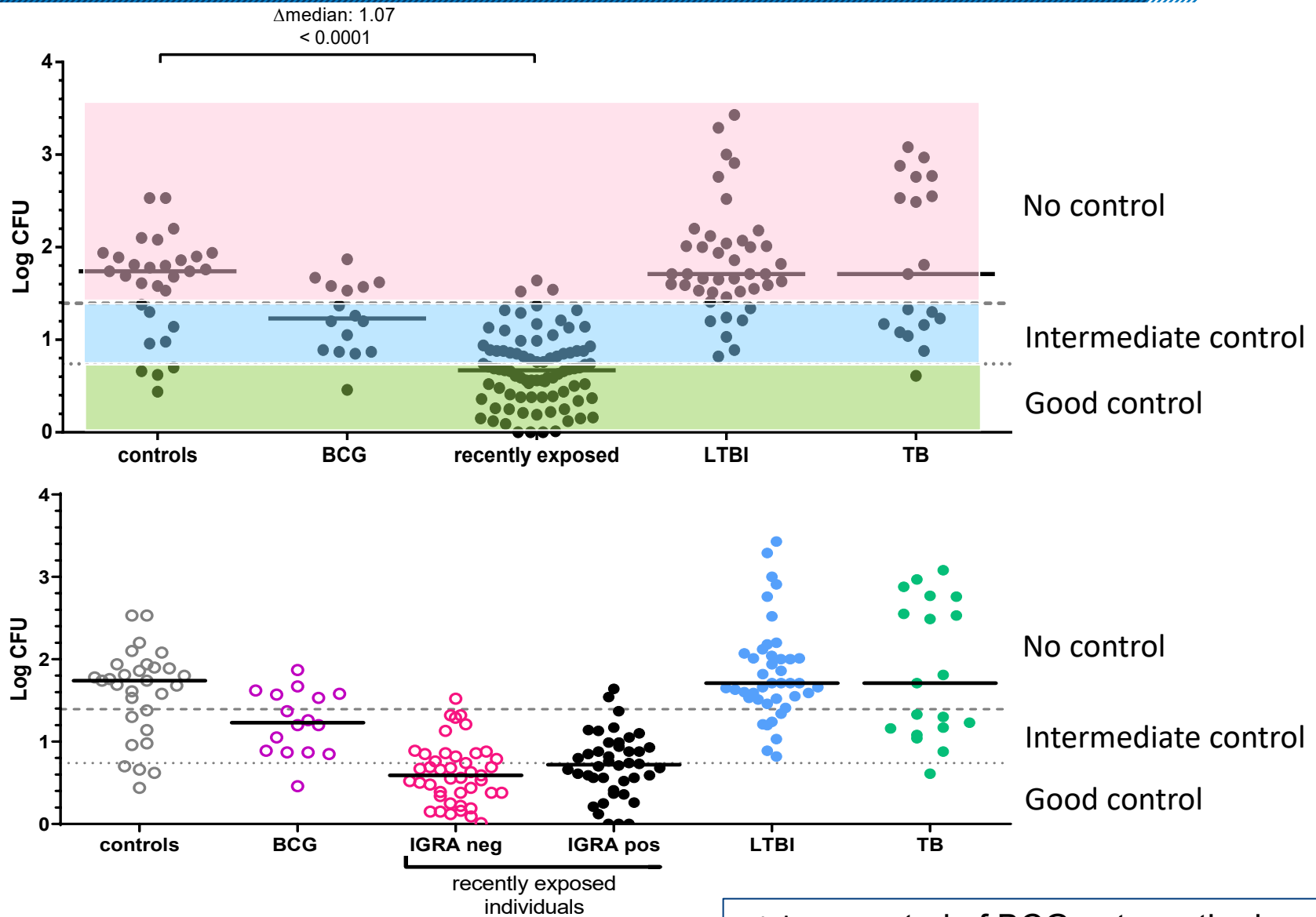
Hoft et al, J Infect Dis. 2002;186(10):1448-57

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

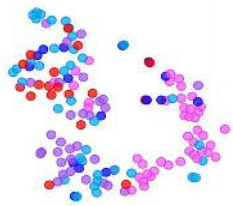
# Strong BCG growth control upon recent exposure



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

# T-SNE identifies CD14dim and CXCL10 in growth control

Group

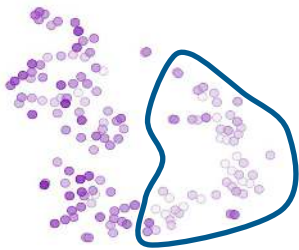


- BCG
- HC
- LTBI
- TB
- exposed

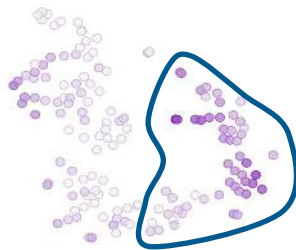
MGIA result



logCFU



ML ratio



CD3



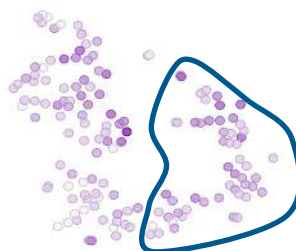
CD4 multifunctional



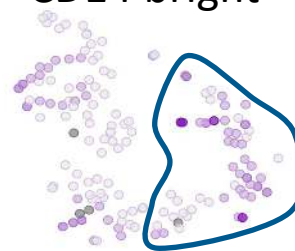
CD8



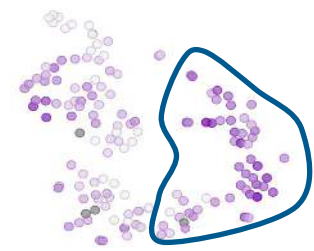
CD19



CD14 bright



CD14 dim



IFN $\gamma$



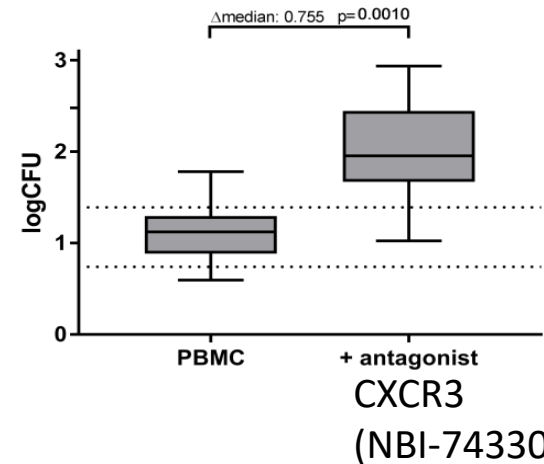
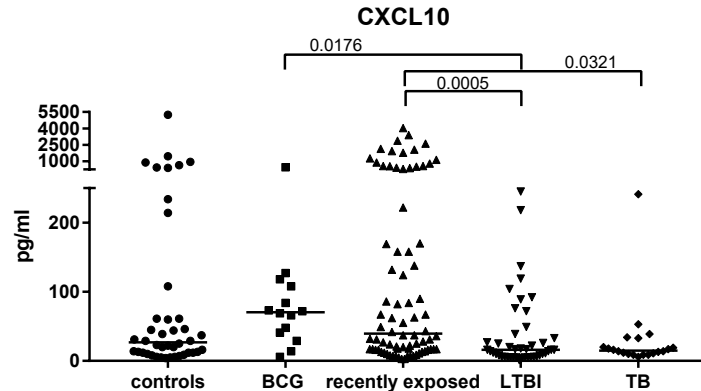
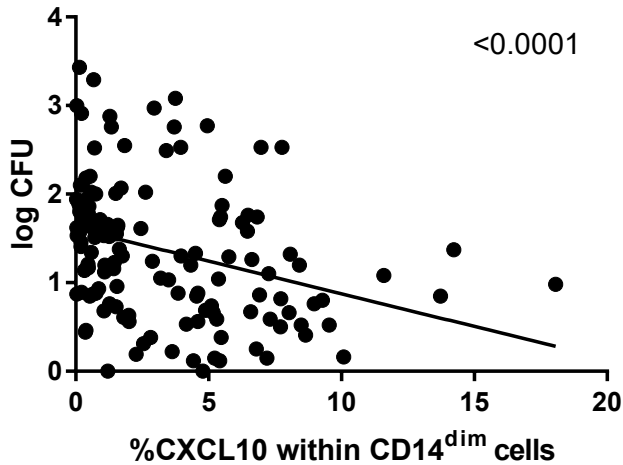
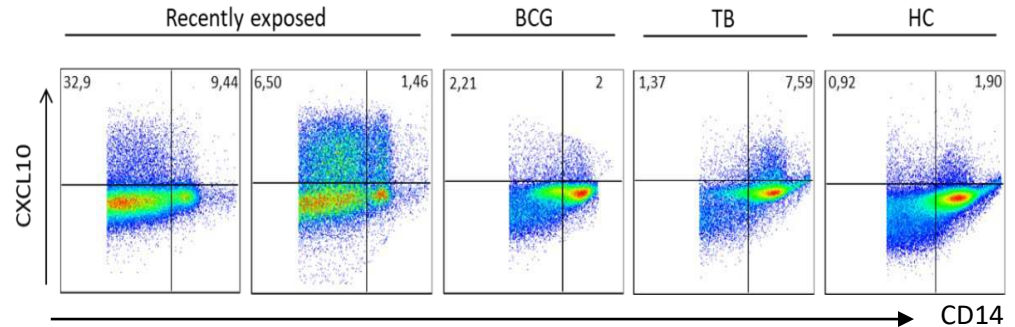
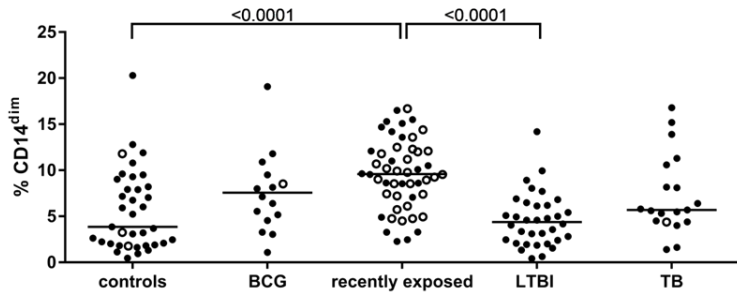
CXCL10



TNF $\alpha$

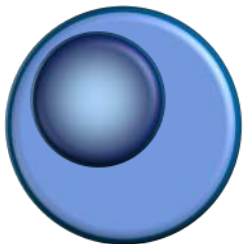


# Non classical monocytes produce CXCL10 that mediates control



CD14dim monocyte: **Non classical monocytes**

Typically express CD16 (FcγRIII) > important in phagocytosis, associated with anti-viral response



## BCG vaccination in humans inhibits systemic inflammation in a sex-dependent manner

Valerie A.C.M. Koeken,<sup>1,2</sup> L. Charlotte J. de Bree,<sup>1,2,3,4</sup> Vera P. Mourits,<sup>1,2</sup> Simone J.C.F.M. Moorlag,<sup>1,2</sup> Jona Walk,<sup>1,2,5</sup> Branko Cirovic,<sup>6</sup> Rob J.W. Arts,<sup>1,2</sup> Martin Jaeger,<sup>1,2</sup> Helga Dijkstra,<sup>1,2</sup> Heidi Lemmers,<sup>1,2</sup> Leo A.B. Joosten,<sup>1,2</sup> Christine S. Benn,<sup>3,4</sup> Reinout van Crevel,<sup>1,2</sup> and Mihai G. Netea<sup>1,2,6</sup>

<sup>1</sup>Radboud Center for Infectious Diseases and <sup>2</sup>Department of Internal Medicine, Radboud University Medical Center, Nijmegen, Netherlands. <sup>3</sup>Bandim Health Project, OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark. <sup>4</sup>Danish Institute for Advanced Study, University of Southern Denmark, Odense, Denmark. <sup>5</sup>Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands. <sup>6</sup>Quantitative Systems Biology, Life and Medical Sciences Institute (LIMES), University of Bonn, Bonn, Germany.

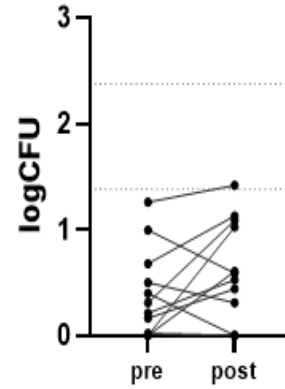
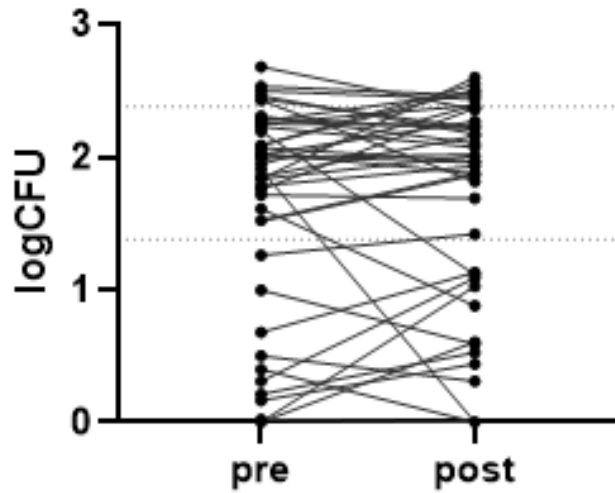
### 300BCG study Nijmegen:

- 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-1 $\beta$  production as marker of trained immunity.

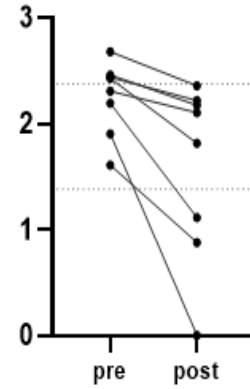
- MGIA
- scRNAseq unstimulated/ LPS stimulation

# MGIA control may exist already before BCG vaccination



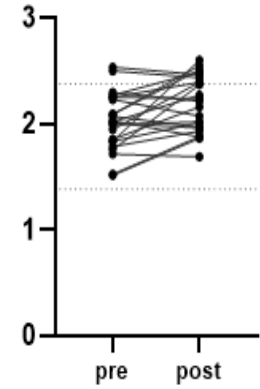
already

26%



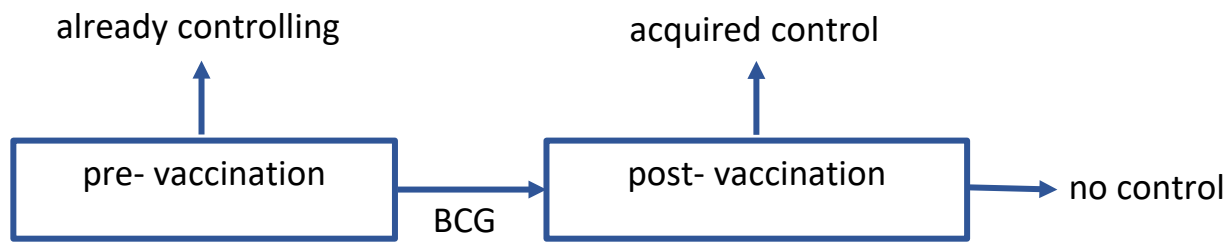
acquired

19%



no control

55%



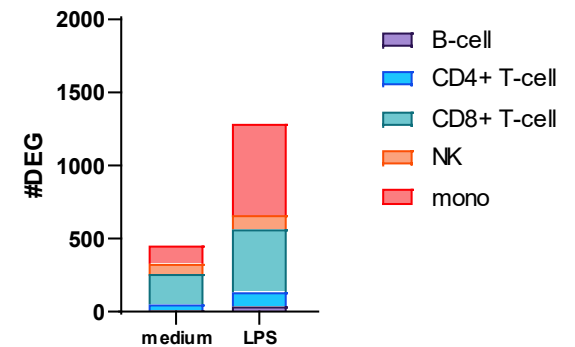
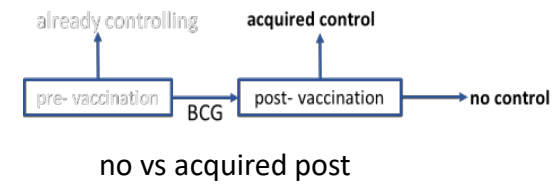
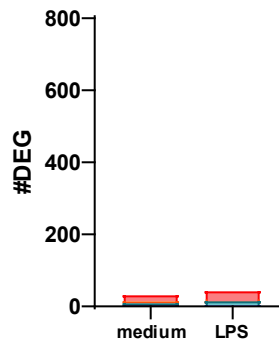
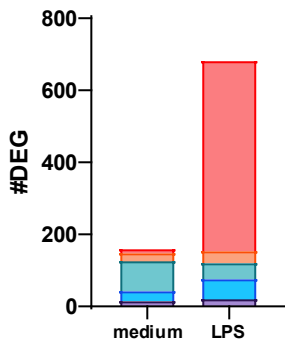
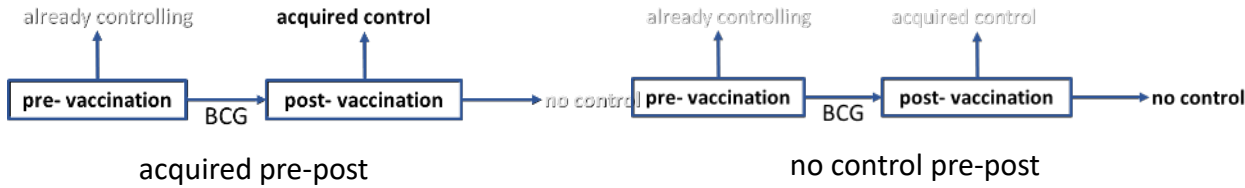
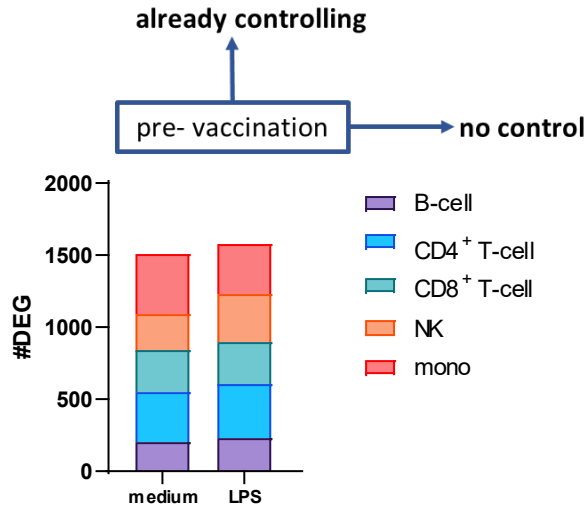
**MGIA control definition:**

**control:**  
pre vacc logCFU < 1,38 (= -1log to v mean inoculum)  
or  
 $\Delta \log CFU_{[V3-V1]} < -0.17$   
(SD mean inoculum)

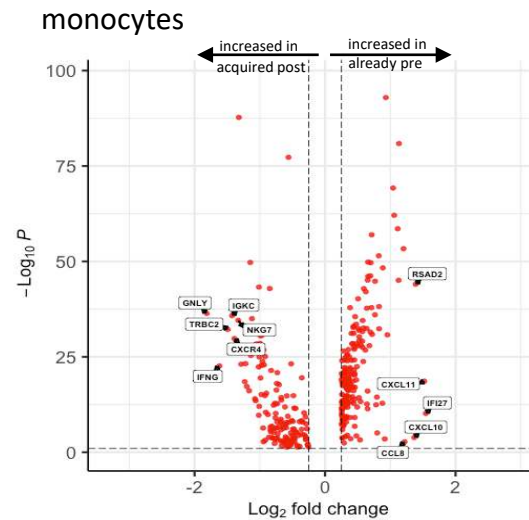
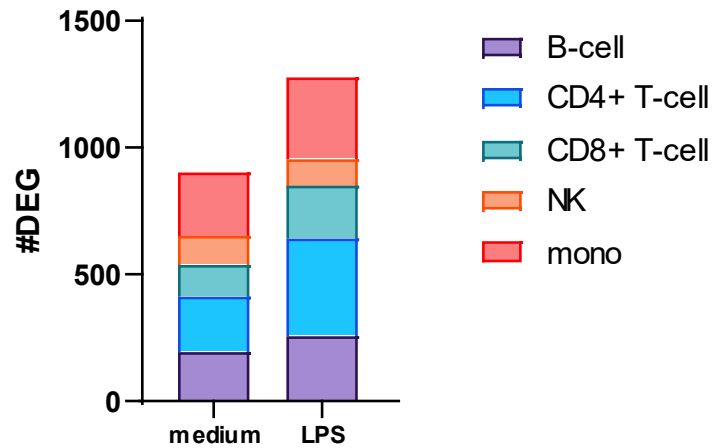
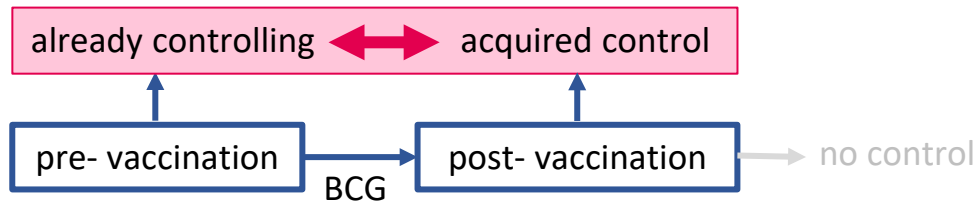
**no control:**  
 $\Delta \log CFU_{[V3-V1]} > -0.17$



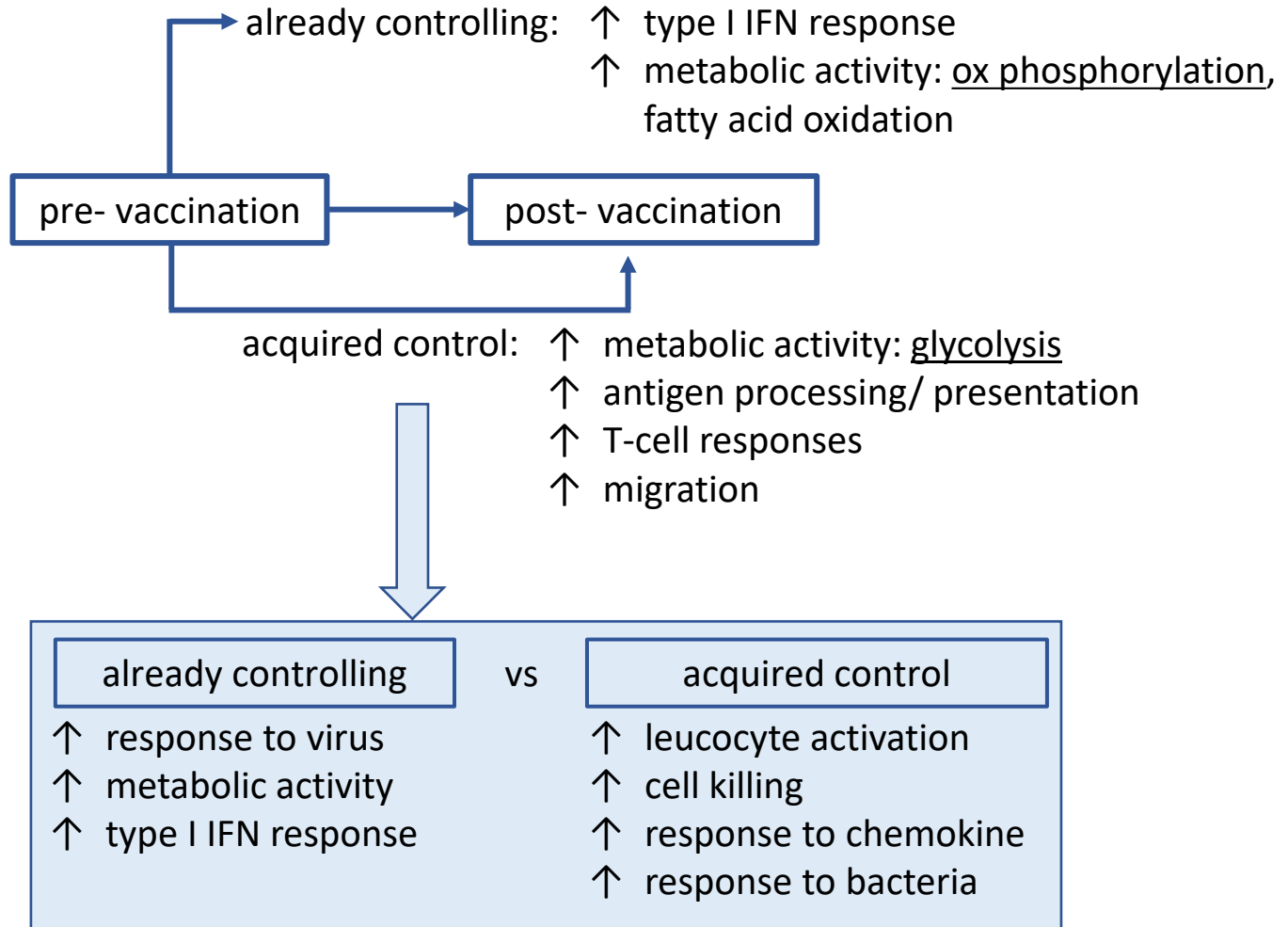
# Differential gene expression in relation to control



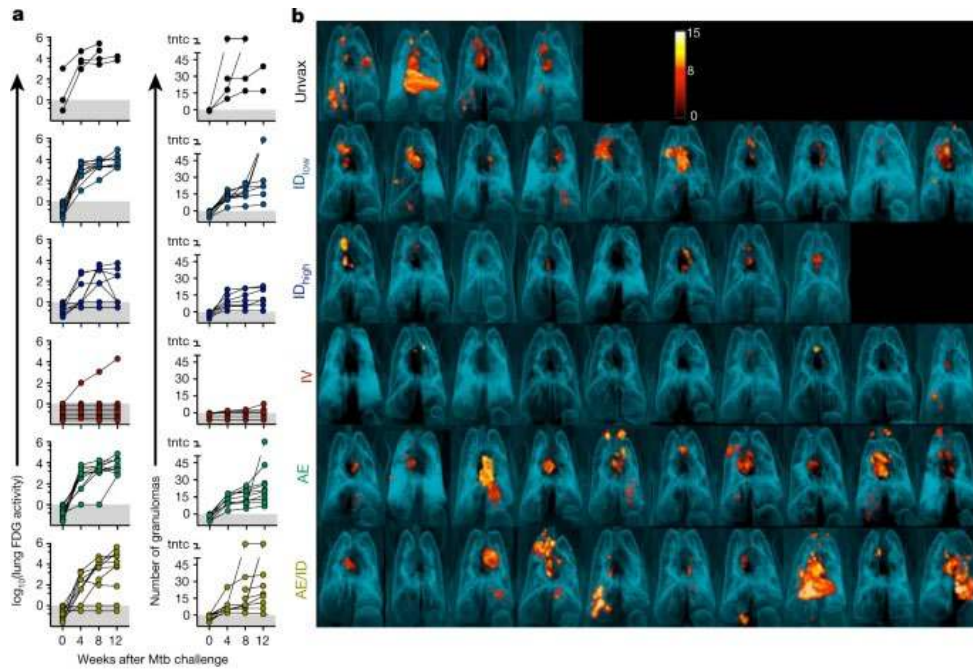
# Already vs acquired samples are different



# Summary – already vs acquired control

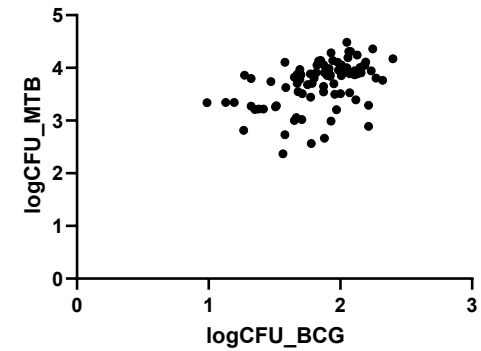


# Growth inhibition assays and protection



IV BCG protects against MTb challenge

(Darrah et al, Nature 2020)



Spearman  $<0.0001$

# Summary

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 Wolfswinkel  
Linda Voogd  
Amy de Waal  
Simone A Joosten  
Tom HM Ottenhoff

VRC, NIAID, NIH, USA

Patricia Darrah  
Mario Roederer  
Bob Seder

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

Infectious Disease Unit, St. John's Research Institute, Bangalore, India

Annapurna Vyakarnam

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



