Immunometabolic profiling of immune cells in tuberculosis patients

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1. Introduction

Aim In this study, we aim to examine immunometabolic profiles of peripheral blood mononuclear cells (PBMCs) isolated from the blood of TB patients.

Background: *M. tuberculosis* is a global pathogen that kills over 1 million people in a year. One in four people are infected with *Mycobacterium tuberculosis* (*Mtb*). Immune cell effector functions are known to be determined by metabolism (Collins *et al*., 2021). *Mtb* infection decelerates bioenergetic metabolism in macrophages and limits the appropriate immune response (Cumming *et al*., 2018). The host immune response is therefore critical in controlling TB infection. The role of metabolism in TB pathogenesis is understudied and remains elusive.

Hypothesis: Metabolic reprogramming of host immunity by *M. tuberculosis* contributes to TB disease

Significance:
Knowledge of the immunometabolic modulations in TB:

- could lead to the discovery of novel prognostic and/or diagnostic biomarkers that will advance therapy and early diagnosis;
- will help inform HDT strategies;
- will allow us to comprehensively study the complete clinical spectrum of TB disease (Figure 4)

2. Materials and Methods

PBMCs will be isolated from the blood of healthy donors and infected with luminescent *Mtb* H37Rv (MOI of 0.1).

Bioelectrospray: *Mtb* infected cells will be encapsulated in microspheres of collagen-alginate using a Nisco electrostatic cell encapsulator (Tezera *et al*., 2017). The microspheres will be cultured at 37 °C in complete RPMI (1640) supplemented with 25 mg/ml kanamycin, 10% Fetal bovine serum and 2mM L-glutamine.

Measurement of Mtb growth: *Mtb* growth will be measured longitudinally by luminescence (Figure 1) and colony forming units (CFU) on Middlebrook 7H11 agar.

***Immune profiling*:** Flow cytometry will be used to identify immune cell populations and to determine the expression of metabolic regulators, glucose transporters, OXPHOS markers (complexes I-V) and pentose phosphate pathway (PPP) markers in these cells (Figure 2 and 3).

3. *Mtb* Luminescence

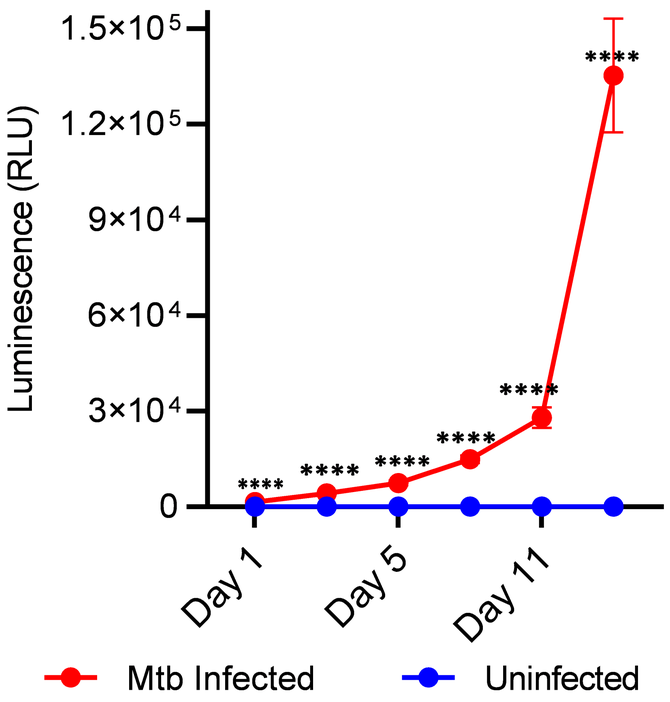


Fig 1: *M. tuberculosis* growth in microspheres monitored by bacterial luminescence, demonstrating the typical *M. tuberculosis* luminescence kinetics of infected PBMCs within microspheres (red). Uninfected PBMCs in the microspheres do not luminesce (blue). **** *p< 0.0001*

4. Identification of immune cell subsets and Complex I-V expression

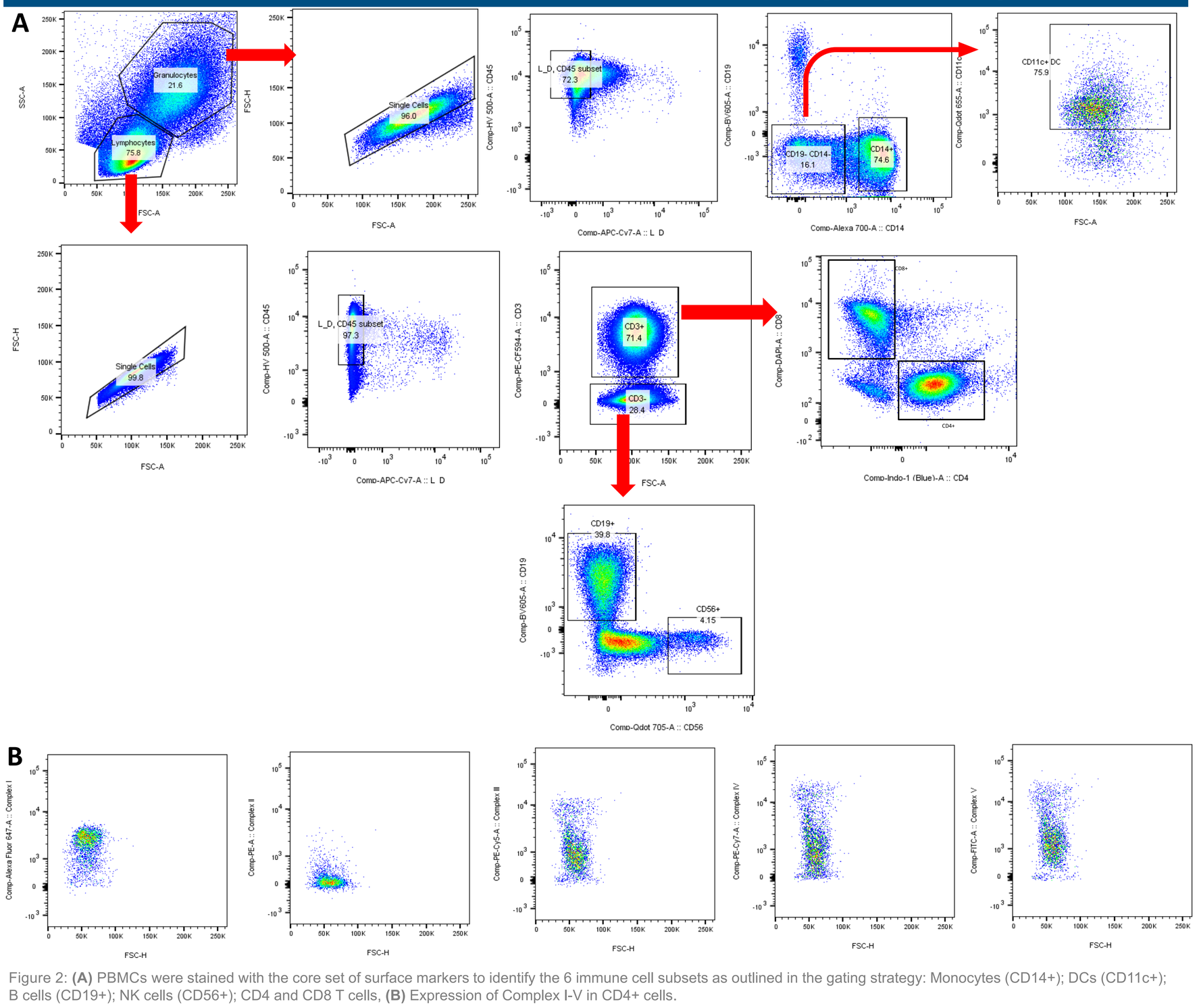
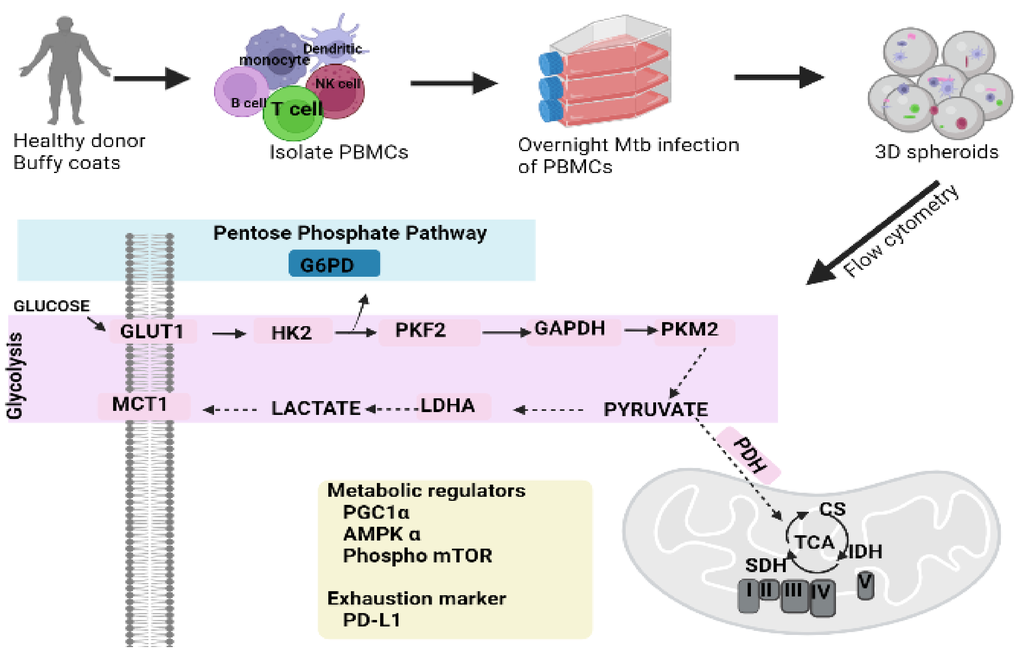
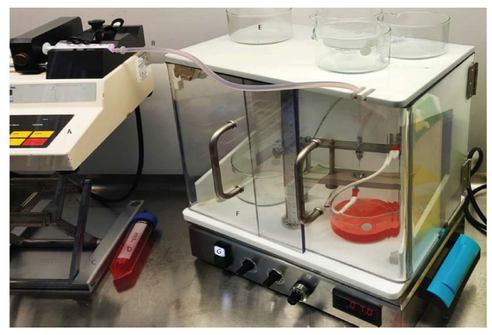


Figure 2: **(A)** PBMCs were stained with the core set of surface markers to identify the 6 immune cell subsets as outlined in the gating strategy: Monocytes (CD14+); DCs (CD11c+); B cells (CD19+); NK cells (CD56+); CD4 and CD8 T cells, **(B)** Expression of Complex I-V in CD4+ cells.

5. 3D cell culture





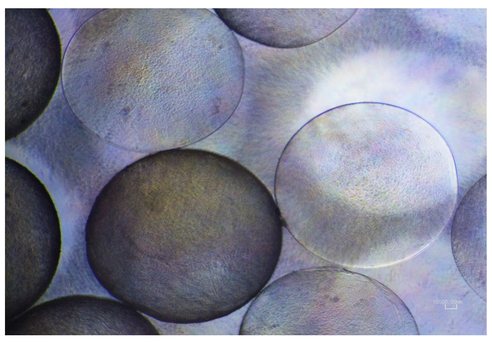


Fig 3: Flow diagram of 3D cell culture assay

6. Immune Profiling of TB patients

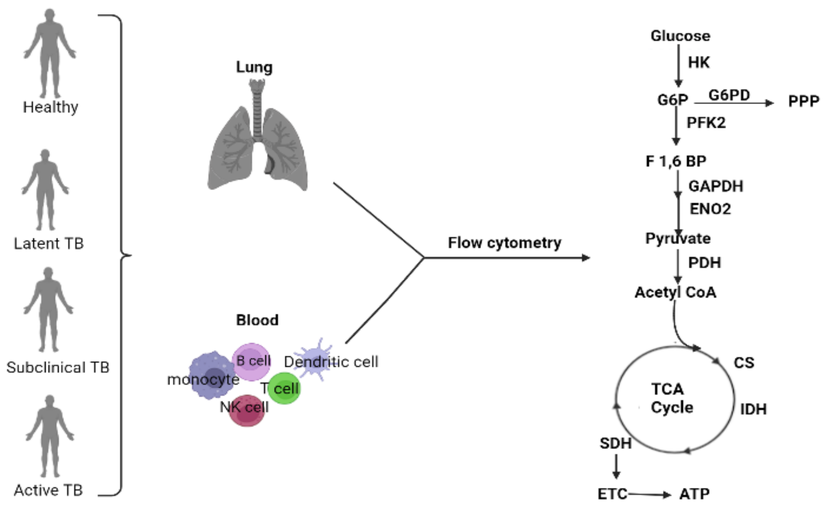


Fig 4: Immune metabolic profiling of immune cells of TB patients

7. Summary

- Host immunometabolism profiling of circulating immune cells in subclinical TB patients may be the key to understanding and improving prediction of TB progression.
- This may lead to a prognostic biomarker which may advance towards prompt therapy and early diagnosis to implement preventative TB therapy

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- SANTHE

References

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