SARS-CoV2 co-infection modulates immunometabolism of *Mycobacterium tuberculosis*-infected human macrophages

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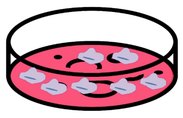
1. Introduction

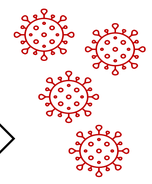
- Tuberculosis caused by *Mycobacterium tuberculosis (Mtb)* is a global health issue
- Globally, sub-Saharan Africa carries the most severe burden
- High incidence rates in South Africa could be exacerbated by high HIV burden and emergence of drug-resistant *Mtb* strains
- The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus2 (SARS-CoV2) has also compounded efforts to eradicate tuberculosis
- A *Mtb*-SARS-CoV2 co-infection model in primary human monocyte-derived macrophages (hMDM) was explored
- SARS-CoV2 strains tested were from the first, second and third wave that affected South Africa

2. Methods



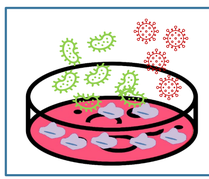
Monocytes isolated from buffy coats were terminally differentiated into macrophages with GM-CSF for 7 days

4-hour *Mtb* H37Rv infection MOI: 1



24-hour SARS-CoV2 infection MOI: 0.1

1st wave: 1313
2nd wave: 5499
3rd wave: d134

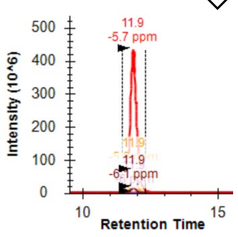


Co-infected hMDM

Supernatant fluid

Cultured in $^{13}C_6$-glucose

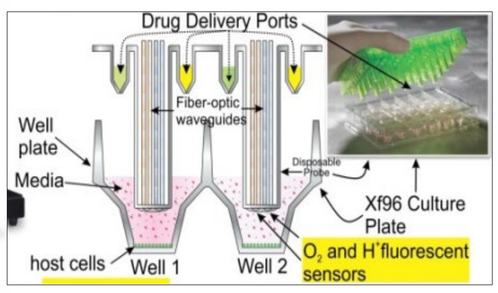
Bio-Plex analysis of supernatant fluid after co-infections using the Bio-Rad Pro Human Cytokine 27-plex assay



Metabolites were extracted with methanol and water (1:1) lyophilized, then reconstituted in water

Metabolites were subject to LC-MS/MS in the Thermo Scientific Q-exactive mass spectrometer

Agilent Seahorse
XFe96 Cell Mito Stress Test analysis



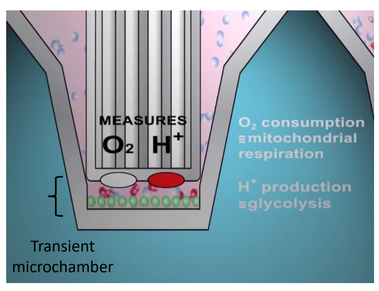


Fig 1. hMDMs were terminally differentiated and infected first fwith Mtb *H37Rv followed by infection with one of three strains of SARS-CoV2. SARS-CoV2 isolates were from the 1st wave (1313), second wave (5499) and 3rd wave (d134). Infected cells were subject to XFe96, metabolite and cytokine analysis.*

3. Detection of cytokines from the supernatant fluid of co-infected hMDMs

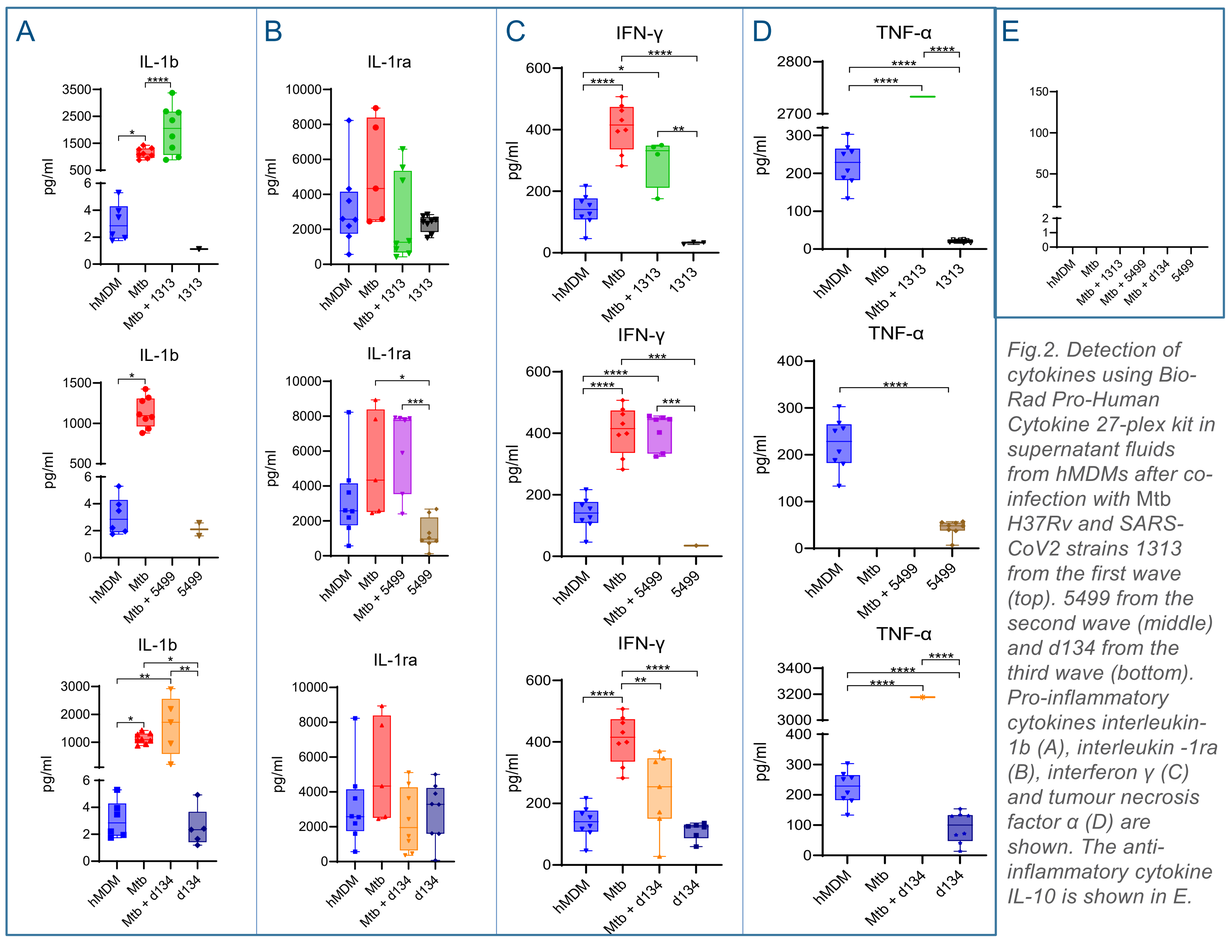


Fig.2. Detection of cytokines using Bio-Rad Pro-Human Cytokine 27-plex kit in supernatant fluids from hMDMs after co-infection with Mtb *H37Rv and SARS-CoV2 strains 1313 from the first wave (top). 5499 from the second wave (middle) and d134 from the third wave (bottom). Pro-inflammatory cytokines interleukin-1b (A), interleukin -1ra (B), interferon γ (C) and tumour necrosis factor α (D) are shown. The anti-inflammatory cytokine IL-10 is shown in E.*

4. Extracellular flux analysis of co-infected hMDMs

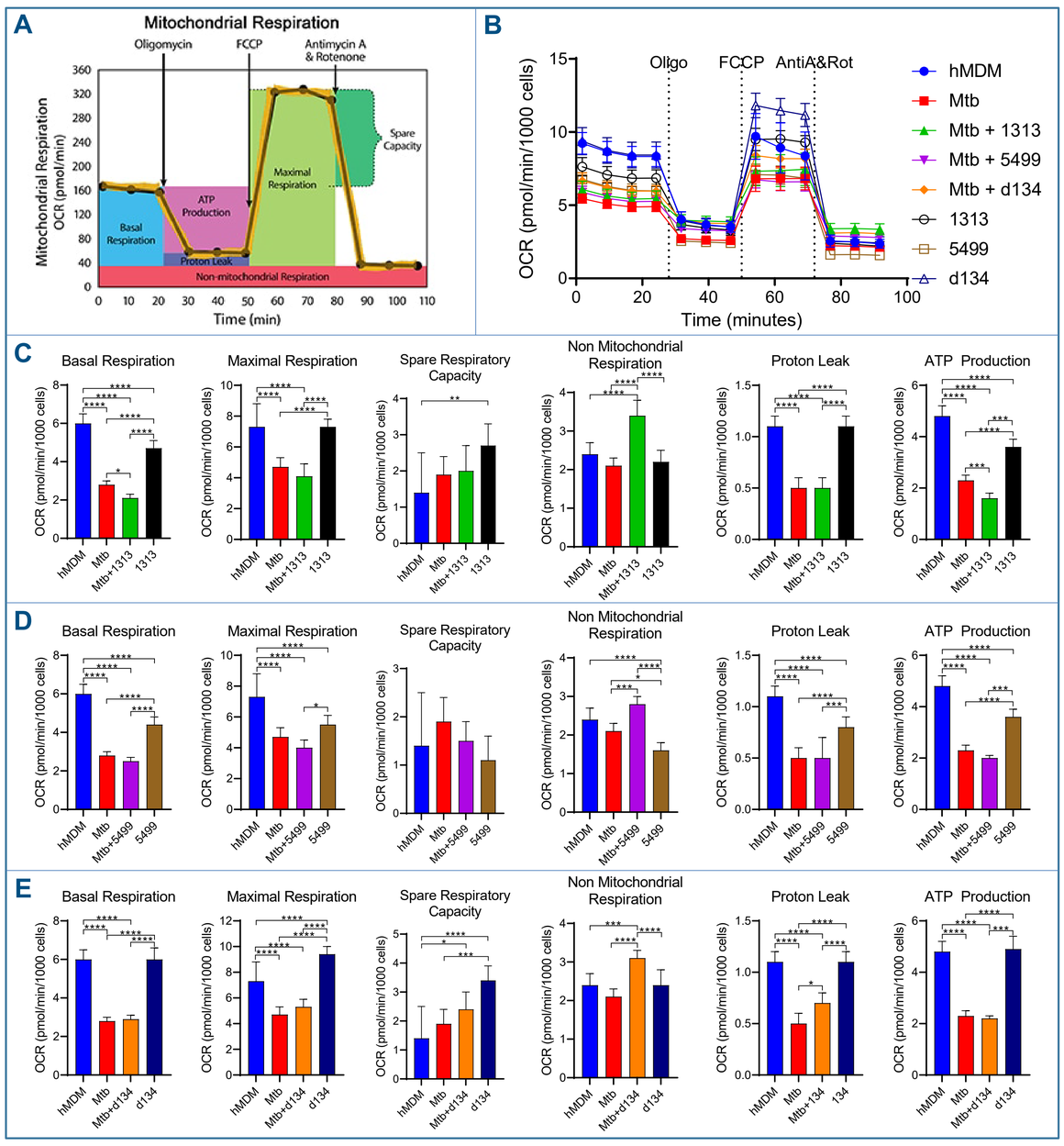


Fig.3 Respiratory profiles and parameters of hMDMs co-infected with Mtb *H37Rv MOI 1 followed by infection with SARS-CoV2 strains 1313, 5499 or d134 at MOI 0.1. A) Typical Cell Mito Stress Test (CMST) profile. B) CMST profile of co-infected hMDMs. Respiratory parameters of hMDMs infected with* Mtb *followed by co-infection with SARS-CoV2 isolate 1313 from the 1st wave (C), 5499 from the 2nd wave (D) and d134 from the 3rd wave (E).*

5. Central carbon tracing of metabolic intermediates

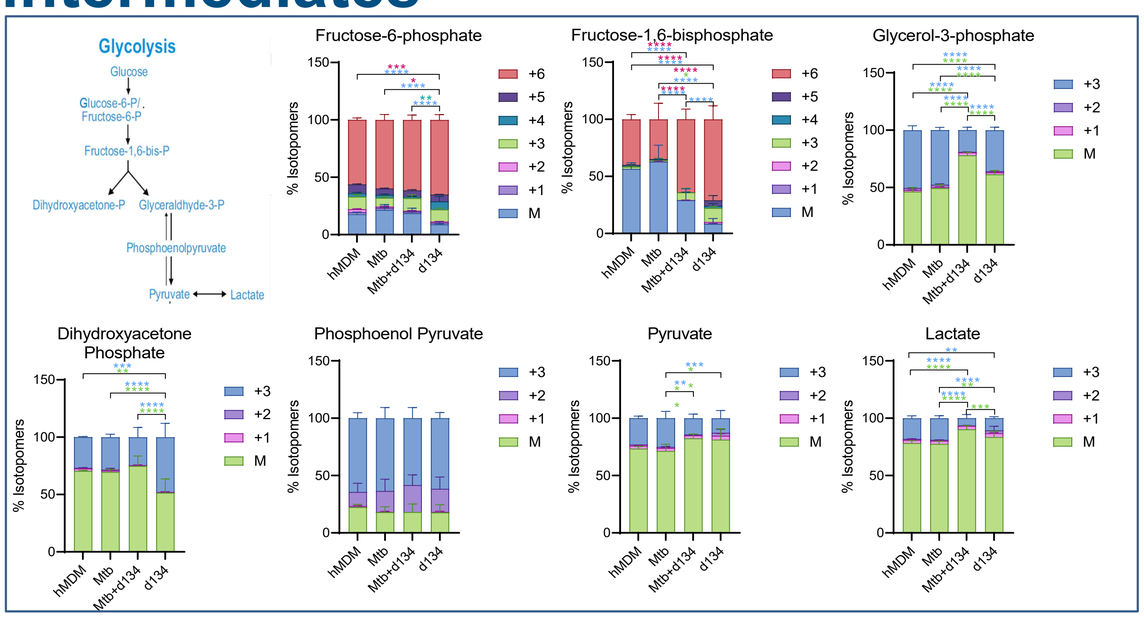


Fig 4 Central carbon tracing of glycolysis intermediates in hMDMs infected with Mtb *H37Rv and SARS-CoV2 d134 cultured in $^{13}C_6$-glucose, and analysed by LC-MS/MS*

Conclusions

1. Co-infection of hMDMs with *Mtb* and SARS-CoV2 induces a pro-inflammatory response with a significant increase in IFNγ levels, when compared to limited IFNγ production in SARS-CoV2-infected hMDMs.
2. Production of IL-10 indicates a significant anti-inflammatory response in hMDMs infected with *Mtb,* and hMDMs co-infected with *Mtb* and either 5499 or d134.
3. Infection with SARS-CoV2 alters the bioenergetic metabolism of *Mtb* H37Rv-infected primary human macrophages in a strain-dependent manner.
4. The bioenergetic parameters in the Cell Mito Stress Test revealed significant decreases in the bioenergetic parameters in the *Mtb*-infected and *Mtb* and SARS-CoV2 infected hMDMs compared to the uninfected hMDMs.
5. Tracing of $^{13}C_6$ glucose suggests infection with d134 increases glycolysis in *Mtb* infected hMDMs, which could influence a pro-inflammatory response

Acknowledgements:
- AHRI Steyn lab
- Professor Alex Sigal, Dr Shi-Hsia Hwa, Dr Sandile Cele and Khadija Khan

Supported by NIH grants R01AI111940, R01AI137043, R61/33AI138280, R01AI134810, and R21A127182 and National Institute of Allergy and Infectious Diseases grant R01AI134810; Bill and Melinda Gates Foundation award OPP1130017; pilot funds from the University of Alabama at Birmingham Center for AIDS Research, Center for Free Radical Biology, and Infectious Diseases and Global Health and Vaccines Initiative. The research was also co-funded by CRDF Global, the South African Medical Research Council, and a South African National Research Foundation Brazil, Russia, India, China, and South Africa multilateral grant.