Mycobacterium tuberculosis infection decelerates bioenergetic metabolism and alters mitochondrial substrate preferences in the macrophage host

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Introduction

- About $^{1}/_{3}$ of the world's population is latently infected with *Mycobacterium tuberculosis* (*Mtb*)[1].
- Infection with *Mtb* does not confer immunity against future infections demonstrating *Mtb* has definitive strategies to manipulate the immune response and prevent complete eradication.
- At the onset of tuberculosis infection, macrophages phagocytose *Mtb* as the first line of defence. Several studies have indicated that *Mtb* infection of murine bone marrow derived macrophages results in a shift to aerobic glycolysis that induces the production of pro-inflammatory cytokines[2,3].
- To further our understanding of *Mtb* modulation of the macrophage to consider host-directed adjunctive therapy as a novel intervention against TB, we investigated how *Mtb* tempers the bioenergetic metabolism of **human monocyte derived macrophages** using extracellular flux and metabolite analyses.

Methods

- Human monocyte derived macrophages (hMDMs) were infected with *Mtb* H37Rv, *M. bovis* BCG (BCG) or heat killed *Mtb* (ΔDead *Mtb*) at multiplicities of infection (MOI) of 1, 2.5 or 5 (as indicated) for 24 hrs.
- The infected macrophages were analysed for their:
  - Overall bioenergetic status using extracellular flux technology (Agilent Seahorse XF96).
  - Metabolite levels and isotopomers after incubation with [U-^{13}C]glucose using LC-MS/MS and ^{13}C-tracing.

Mtb infection modulates the bioenergetic phenotype of macrophages distinct to that of BCG and heat killed *Mtb* infections

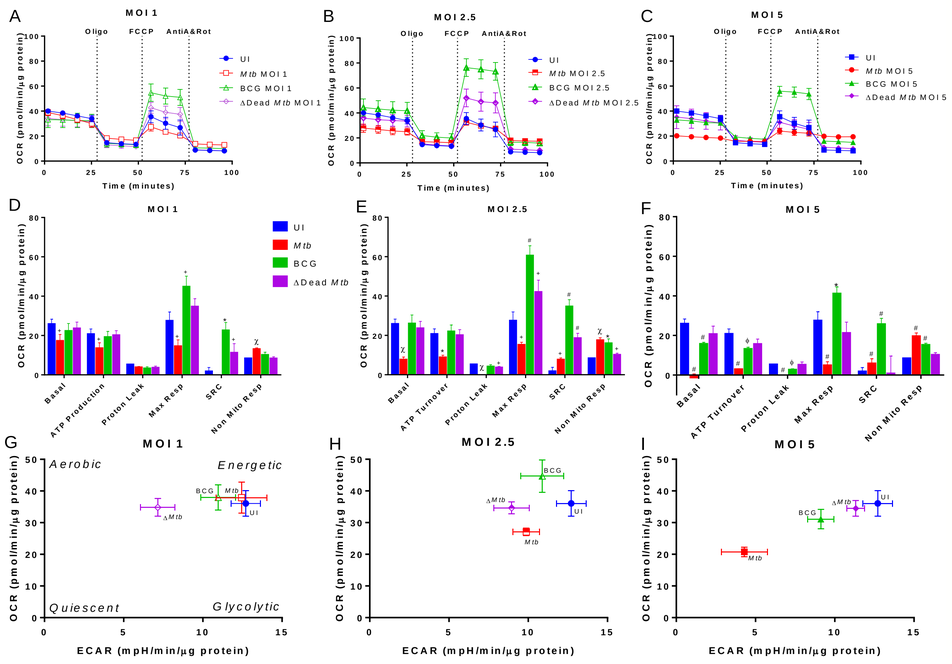

Figure 1. Increasing MOI of *Mtb* depresses mitochondrial respiration in hMDM in contrast to BCG infection, which increases the spare respiratory capacity of the hMDMs. (A-C) **Cell Mito Stress Test** (CMST) of hMDM infected with increasing MOI of *Mtb*, BCG and heat-killed *Mtb for* 24 hrs. (D-F) Respiratory parameters of the macrophages calculated from the CMST. (G-I) Phenograms demonstrating a shift in *Mtb* infected hMDMs towards an energy phenotype reminiscent of quiescence at an MOI of 5.

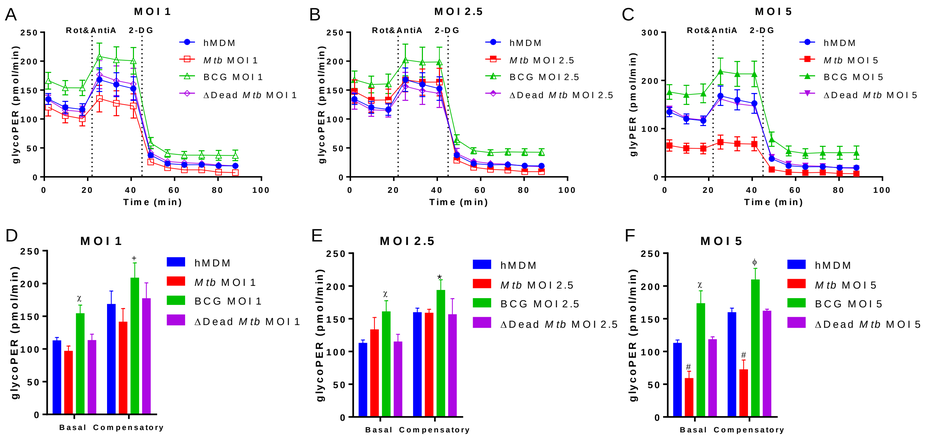

Figure 2. Unlike BCG infection, *Mtb does not* increase the rate of glycolysis in hMDMs. (A-C) **Glycolytic Rate Assay** of hMDM infected with increasing MOI of *Mtb*, BCG and heat-killed *Mtb* for 24 hrs. (D-F) Basal Glycolysis and compensatory glycolysis of the macrophages expressed as proton efflux rate (PER, pmol/min) as determined from the Glycolytic Rate Assay on the XF96. The glycolytic proton efflux rate gives a direct measure of the acidification rate due to glycolysis without the acidification contribution from mitochondrial respiration.

Mtb infection decelerates bioenergetic metabolism of the macrophage

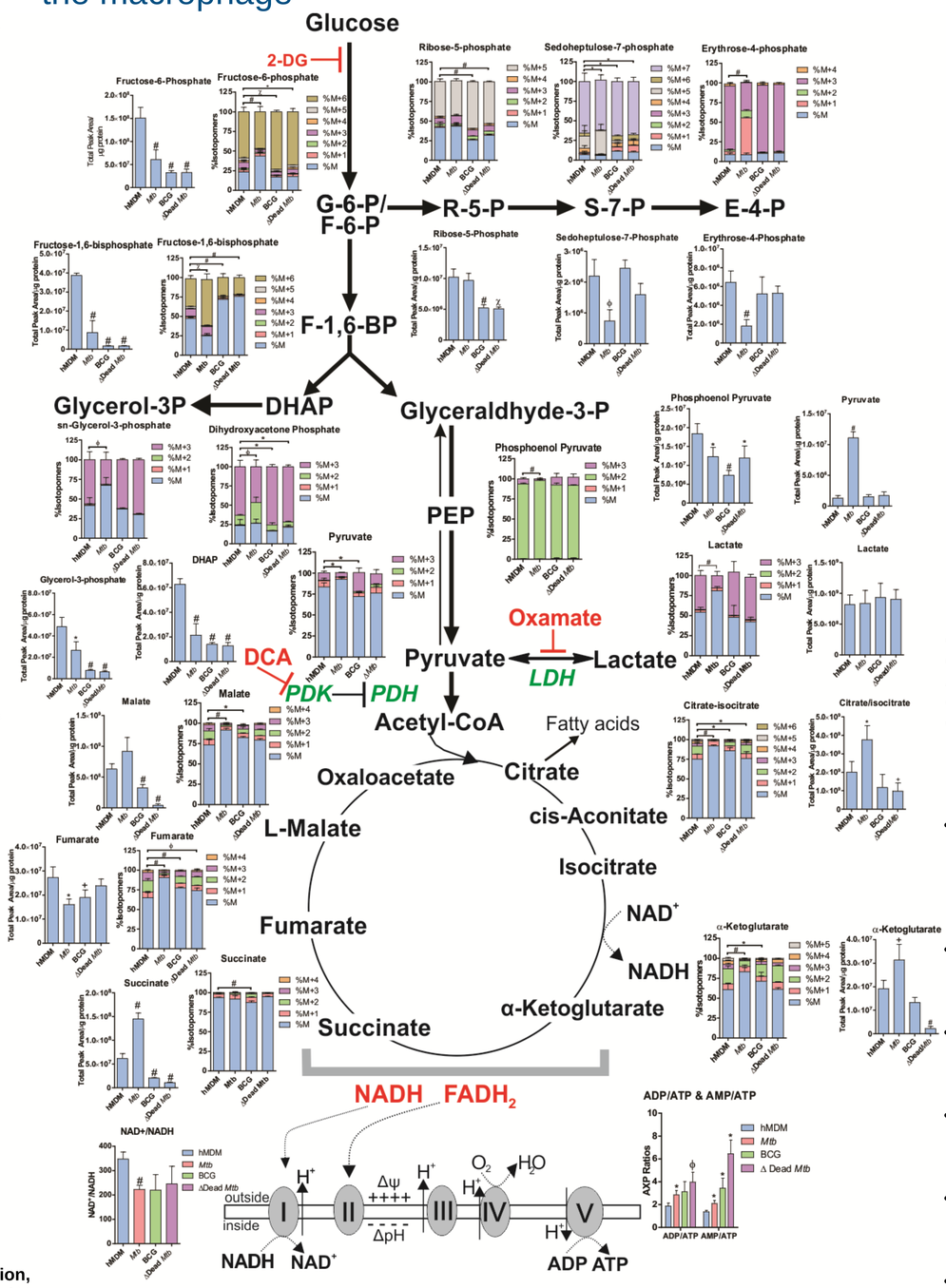

Figure 3. *Mtb* infection decelerates bioenergetic metabolism in hMDMs. ^{13}C-tracing of metabolites extracted from hMDM infected with *Mtb*, BCG or heat-killed *Mtb* at an MOI of 4 for 24 hours. Less ^{13}C incorporation is observed in the metabolites of glycolysis, TCA cycle and the pentose phosphate pathway of the *Mtb* infected hMDMs in comparison to the uninfected hMDMs and those that are infected with BCG and heat-killed *Mtb*. Although the levels of the glycolytic intermediates are decreased in all of the infected hMDMs, the levels of the TCA cycle intermediates from *Mtb* infected hMDMs are higher possibly due to lack of cycling through the TCA cycle.

Inhibition of glycolysis in *Mtb* infected macrophages does not redirect energy metabolism to OXPHOS

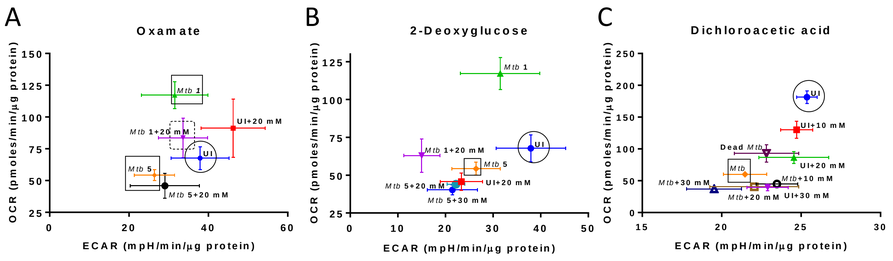

Figure. 4. Inhibition of glycolysis (2-deoxyglucose) or lactate production (oxamate and dichloroacetic acid) do not redirect the bioenergetic metabolism of *Mtb* infected macrophages towards OXPHOS. Phenograms of uninfected and *Mtb* (1 or 5: MOI) hMDM cells treated with (A) oxamate (B) 2-deoxyglucose (C) and RAW264.7 cells (*Mtb*, MOI 5) treated with dichloroacetic acid. Circles: uninfected macrophages, Squares: *Mtb* infected macrophages.

Mtb infection alters mitochondrial substrate preference of the macrophage

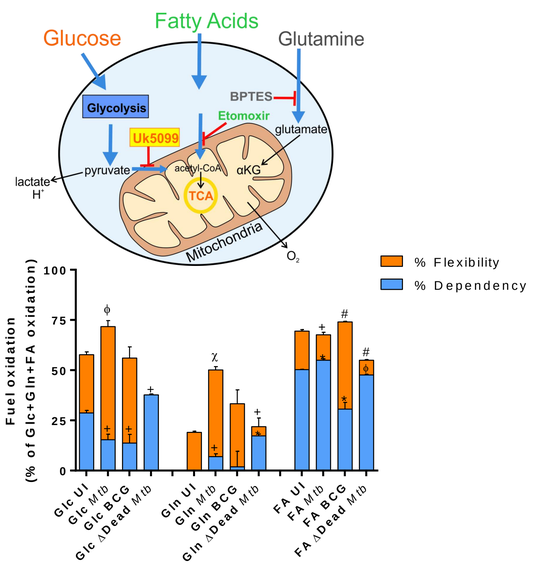

Figure 5. *Mtb* infection decreases the mitochondrial dependency on glucose and increases the dependency on glutamine and fatty acid oxidation. hMDM were infected with *Mtb*, BCG and heat-killed *Mtb* (MOI 4) for 24 hrs prior to conducting the Mitochondrial Fuel Flex Test on the XF96. Glc, glucose; Gln, glutamine; FA, fatty acids

Conclusions

- Basal respiration, ATP-linked OCR and maximal respiration are reduced in *Mtb* infected hMDMs with increasing MOI in contrast to infection with BCG and heat-killed *Mtb* that do not affect respiration at low MOIs.
- Furthermore, BCG infection *increases* the maximal respiration and the spare respiratory capacity of hMDMs above that of uninfected hMDMs.
- Unlike BCG or heat killed *Mtb* infection, *Mtb* infection *does not increase* the rate of basal glycolysis or compensatory glycolysis in hMDMs.
- *Mtb* infection decelerates flux through glycolysis and the TCA cycle of the hMDMs, confirming the observed shift to a quiescent energy phenotype.
- ADP/ATP and AMP/ATP ratios increase in *Mtb* infected hMDMs supporting the overserved reduced rates of OXPHOS and glycolysis.
- Inhibition of glycolysis or lactate production did not redirect the bioenergetic metabolism of the macrophage to OXPHOS suggesting that *Mtb* has an irreversible depressive effect on mitochondrial respiration.
- *Mtb* infection of hMDMs changes the substrate preference of mitochondria from glucose to glutamine and fatty acids, whereas heat-killed *Mtb* increases the dependency of the macrophages on glucose with no flexibility.
- *Mtb* infection of hMDMs slows down energy metabolism of the host cell, including both OXPHOS and glycolysis. This is probably part of *Mtb's* strategy to "diminish" the innate immune functions of the macrophage and delay apoptosis of the macrophage to sustain bacilli growth.
- Future work includes investigating the "apoptotic status" of *Mtb* infected hMDMs displaying the quiescent energy phenotype.

References

1. Global TB Report, 2017, WHO.
2. Gleeson et al. (2016) J. Immun. Vol. 196, pp. 2444;
3. Stanley et al. (2016) Vol. 197, pp. 1287

Acknowledgements

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