Distinctive production and usage of glycolytic and mitochondrial metabolism

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Introduction

- Cellular energy metabolism is an essential core process required for immune cell activation. A well-known metabolic response of many immune cells including T cells and macrophages is an immediate increase in glycolytic activity which is followed by further long-term reprogramming of metabolic phenotype.
- Activation of CD4+ T cells is followed by rapid proliferation and differentiation into specific subsets with effector or suppressor functions and ultimately generation of differentiated memory cells. These transitions are accompanied by tight regulated changes in energetic demand and cellular metabolic reprogramming.
- Proinflammatory macrophage activation is accompanied with bi-phasic metabolic reprogramming; the immediate early increase in glycolysis and the later iNOS-dependent suppression of mitochondrial respiration.
- A newly developed a cell-based assay using Agilent Seahorse Extracellular Flux technology enables simultaneous measurement of the two-main cellular ATP-producing pathways, i.e. glycolysis and oxidative phosphorylation. The assay allows for quantification of real-time changes in total ATP production rate, and the fractional contribution of the individual pathways to support bioenergetic demands.
- The ATP energy production rate changes were examined during T cell and murine macrophage activation using this new approach.

Approach

Real-time ATP Production Rate Measurement

The Agilent Seahorse XF Real-Time ATP Rate Assay is a cell-based assay which allows simultaneous measurement of the two-main bioenergetic pathways to calculate the total rate of cellular ATP production as well as the fractional contribution from each pathway.

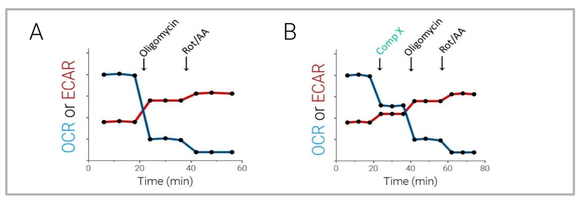

Fig 1. Assay design of Agilent Seahorse XF Real-Time ATP rate assay. ATP production rate can be assessed by serial injection of mitochondrial complex inhibitors, oligomycin and rotenone/antimycin A (Rot/AA) without (A) or with (B) metabolic modulation by a test compound (Comp X).

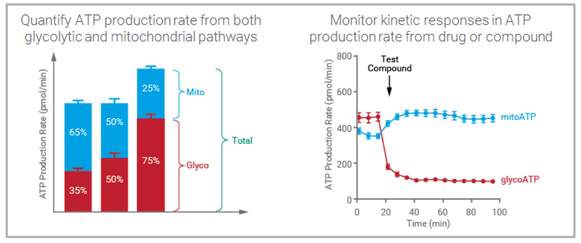

Fig 2. Key outputs of Agilent Seahorse XF Real-Time ATP rate assays. (A) Illustrates quantitative metabolic shift from mitochondrial respiration to glycolysis, resulting in different phenotypes under different conditions. (B) Shows the changes of mitoATP production rates and glycoATP production rates over a time course in one cell population.

Results and Discussion

Glycolysis supports CD4+ T cell activation

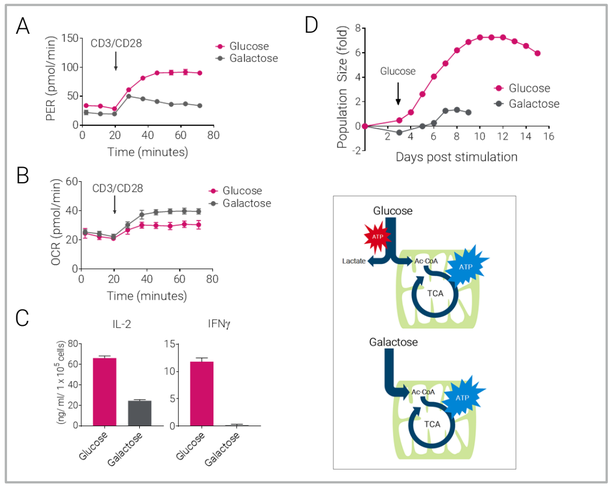

Fig 3. Naïve CD4+ T cells were *in situ* activated by administration of beads conjugated with anti-CD3 and anti-CD28 antibodies in Seahorse XF RPMI Medium, pH7.4 containing10mM glucose or galactose,1 mM pyruvate and 2 mM glutamine. Changes in (A) Proton Efflux Rate (PER) and (B) Oxygen Consumption Rate (OCR) were monitored using an Agilent Seahorse XF Analyzer. (C) Accumulation of IL-2 and IFN-γ were measured in the extracellular medium after 2 days of activation. (D) After 3 days of activation, glucose (10mM) was incorporated in the cell culture medium of galactose-activated T cells and cell proliferation in glucose containing media was monitored up to day 15 post-activation (representative graph of 3 independent replicates).

CD4+ cell expansion after CD3/CD28 activation is sustained by mitochondrial ATP production

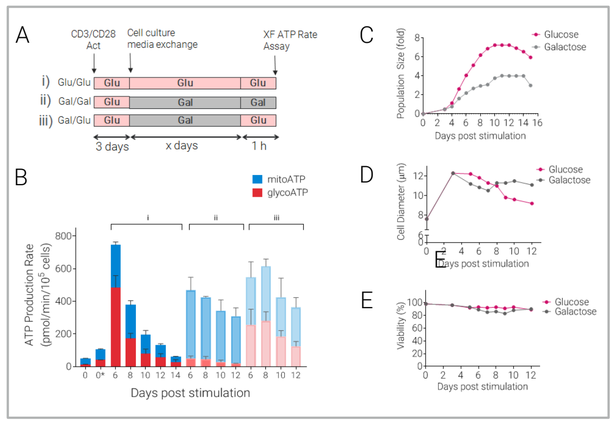

Fig 4. Naïve CD4+ T cells were activated with anti-CD3/anti-CD28 conjugated beads in cell culture medium containing glucose. After 3 days post stimulation, in some of the conditions glucose was replaced by galactose in culture medium and samples were taken every 2 days to analyze (Scheme A). (B) XF Real-Time ATP Rate Assay was performed in samples of activated T cells at indicated days post stimulation. (C) Cell proliferation, (D) cell size profile, and (E) viability of activated T cells expanded in cell culture medium containing 10 mM glucose or galactose.

The role of glucose during CD4+ T cell activation is beyond ATP production

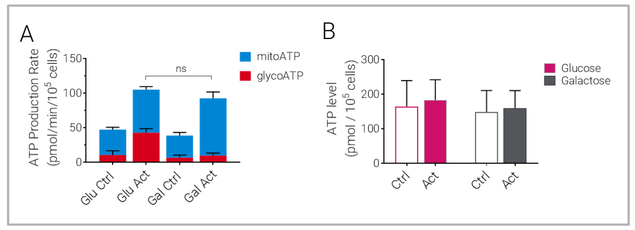

Fig 5. Naïve CD4+ T cells were *in situ* activated by administration of beads conjugated with anti-CD3 and anti-CD28 antibodies in Seahorse XF RPMI Medium, pH7.4 containing 10 mM glucose or galactose, 1 mM pyruvate and 2 mM glutamine (200 K cells/well). After 40 min of bead injection, cells were analyzed for (A) ATP production rate using the Seahorse XF Real-Time ATP Rate Assay or (B) intracellular ATP levels using Cell Titer-Glo® Luminescent Cell Viability Assay (Promega). Graphs represent mean ± SD of 3 independent replicates.

ATP production rate is sustained while metabolic profile changed during macrophage activation

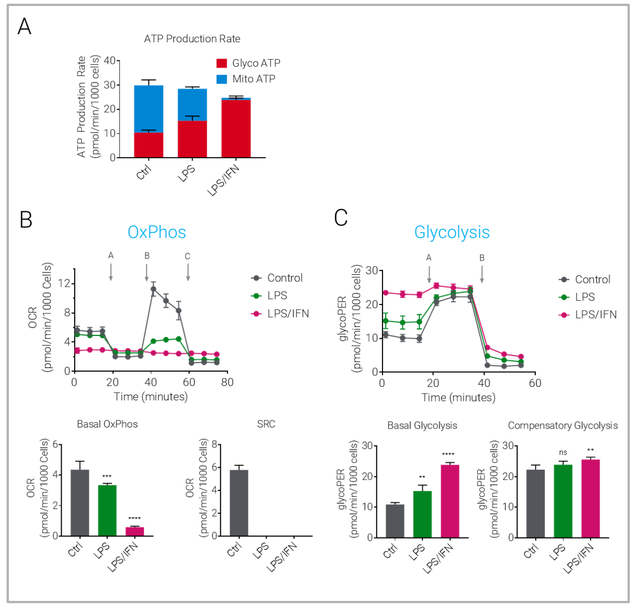

Fig 6. The ATP production rates of RAW264.7 cells were compared with or without overnight stimulation with LPS or LPS together with IFNγ. Cells showed a phenotypic change to more glycolytic phenotype upon the activation while the overall ATP production rates were maintained stably even with severe suppression of mitochondrial respiration by LPS/IFNγ co-stimulation. The further detail metabolic profile changes were examined by Seahorse Mito Stress Test (B) and by Glycolytic Rate Assay (C). Although the basal respiration rate was moderately decreased by LPS alone, but the spare respiratory capacity (SRC) was severely abolished. The compensatory glycolytic rate was not changed significantly while the basal glycolytic rate was increased upon activation as compensating the mitochondrial activity loss. Data shown are mean ± SD, n=6 technical replicates. (*, p<0.05; **, p<0.005; ***, p<0.0005; ****, p<0.00005).

Bi-phasic activation of macrophages

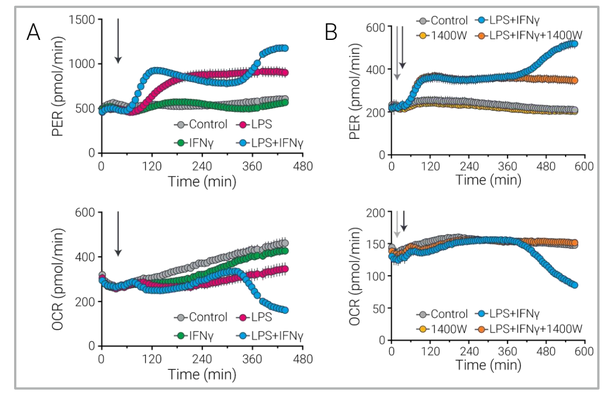

Fig 7. (A) RAW264.7 cells were stimulated by injection of LPS, IFNγ or both, and metabolic responses were measured for an extended period (> 6 h) after the stimulation. Data shown are mean ± SD, n=4 technical replicates. (B) Assay medium with or without iNOS inhibitor (1400W, *gray arrow*) was injected prior to LPS and IFNγ (*black arrow*). Only the secondary changes in PER and OCR were completely blocked by the inhibitor. Data shown are mean ± SD, n=6 technical replicates.

Conclusions

- The Agilent Seahorse XF Real-Time ATP Rate Assay enables a new insight of the role of ATP production form glycolysis and mitochondrial oxidative phosphorylation during T cell differentiation.
- Replacement of glucose by galactose completely blocked CD4+ T cell activation despite no differences in cellular ATP production highlighting the role of glucose metabolism during T cell activation.
- Nutrient availability has a critical role not only during T cell activation but also in subsequent expansion/differentiation being determinant in defining T cell fate and function.
- *In vitro* macrophage activation is tightly associated with the oxidative-to-glycolytic switching in metabolic phenotype mediated by bi-phasic responses; immediate early (< 1h) and prolonged (> 6h), which makes cellular ATP energy production more depend on glycolysis.
- LPS/IFNγ co-stimulation suppresses mitochondrial function severely through iNOS signaling and cells turns to rely on glycolysis fully for the ATP energy production.

References

- www.agilent.com/chem/discoverxf
- www.agilent.com/chem/immunology
- www.agilent.com/chem/realtimeatp