Changes In Metabolic Phenotype And Cellular ATP Production During CD4+ T Cell Activation

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AAI 2018
POSTER # IRC.502

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Introduction

- 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.
- Environmental factors such as nutrient availability, pH and O_2 also have significant impact in the metabolism of immune cells, also affecting immune cell differentiation and function.
- Using Agilent Seahorse Extracellular Flux analysis, we have developed a cell-based assay for 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.
- In this work we explored the influence of glucose availability at different time points over the course of naïve CD4+ T cell activation and differentiation with particular focus on its bioenergetic role.

Experimental Approach

Method

- 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.
- Since mammalian cells exquisitely regulate the ATP production with ATP consumption, measuring the rate of ATP production enables a view into cellular function that is not provided by measuring the amount of intracellular ATP in the cell.

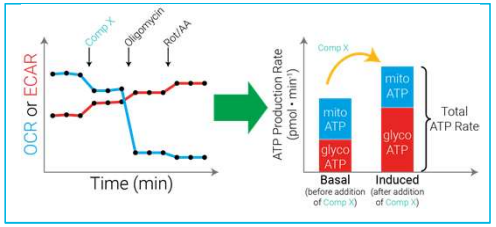

Figure 1. Representative scheme of Agilent Seahorse XF Real-Time ATP Rate Assay. Kinetic profile of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) measurement. OCR and ECAR rates are first measured. Injection of oligomycin results in an inhibition of mitochondrial ATP synthesis that results in a decrease in OCR, allowing mitochondrial ATP production rates to be quantified. Complete inhibition of mitochondrial respiration with rotenone plus antimycin A enables calculation of mitochondrial-associated acidification, allowing calculation of glycolytic ATP production.

Results and Discussion

Glucose but not galactose supports activation of naïve CD4+ T cells.

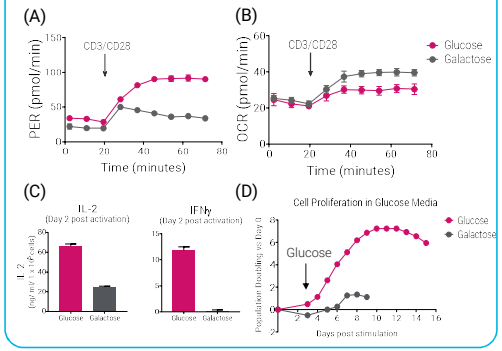

Figure 2. Naïve CD4+ T cells were *in situ* activated by administration of beads conjugated with anti-CD3 and anti-CD28 antibodies in Seahorse XF RPMI Medium, pH 7.4 containing 10 mM glucose or galactose, 1 mM pyruvate and 2 mM glutamine. Changes in (A) Proton Efflux Rate (PER) and (B) Oxygen Consumption 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 (10 mM) 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).

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

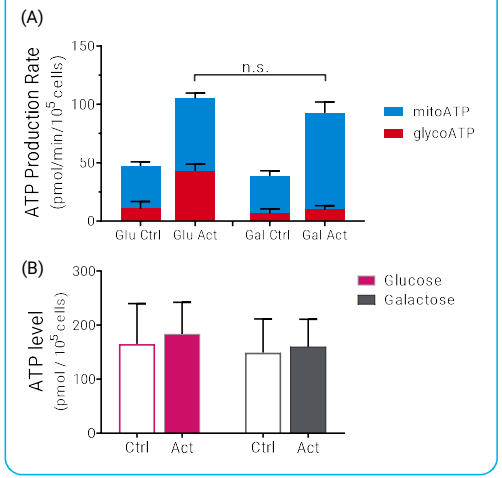

Figure 3. Naïve CD4+ T cells were *in situ* activated by administration of beads conjugated with anti-CD3 and anti-CD28 antibodies in Seahorse XF RPMI Medium, pH 7.4 containing 10 mM glucose or galactose, 1 mM pyruvate and 2 mM glutamine (200K cells/well). After 40 min of beads injection, cells were analyzed for (A) ATP production rate using the Seahorse XF Real-Time ATP Rate Assay or (B) intracellular ATP levels using CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Graphs represent mean ± SD of 3 independent replicates.

Results and Discussion

CD4+ T cell expansion after CD3/CD28 activation is sustained by mitochondrial ATP production in galactose medium

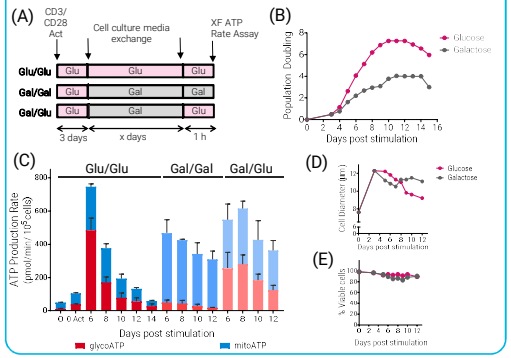

Figure 4. Naïve CD4+ T cells were activated with anti-CD3/anti-CD28 conjugated beads in cell culture medium containing glucose. After 3 days post stimulation, in some of the conditions glucose was replaced by galactose in culture medium and samples were taken every 2 days to analyze (Scheme A). (B) Cell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

Differentiation in galactose media increases differentiation in central memory T cells with minimal effector differentiation

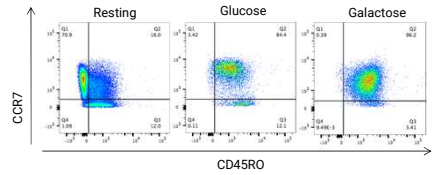

Figure 5. Flow analysis of T cells at Day 8 post stimulation in glucose medium and expanded in glucose or galactose media as indicated in Figure 4A.

Conclusions

- The 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.

References

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