

Measuring Glycolytic Function in Cells

Real-Time, Kinetic Measurement of Glycolysis Connects Energy Pathways to Cell Growth and Proliferation

Application Brief

Introduction

Glycolysis is used by virtually all cell types and is an important cellular function in a variety of research areas. For example, dividing cells rely heavily on glycolysis, and it has been shown to play a significant role in both normal cellular processes such as immune cell activation as well as cellular dysfunction, as occurs in cancer. The methods of studying glycolysis reflect the perpetually changing landscape of biotechnology from microelectrodes, spectrophotometers and NMR, to quantitative metabolic analyzers. As the scientific research questions that involve glycolysis have become more complex, the methods applied to studying glycolysis have become more analytical and relevant to living cells.

As described by the Embden-Meyerhof-Parnas pathway, glycolysis is a series of 10 sequential reactions that break down glucose. The products of these reactions include protons and pyruvate, which may then be converted to lactate. The cosecretion of protons and lactate results in the acidification of the media¹. In this scenario, the glycolytic pathway could be thought of as an engine, while the glucose molecule feeding into the pathway is the fuel. Agilent Seahorse XF technology examines glycolytic activity, or metabolic engines, as well as the substrates or fuels used by measuring the extracellular acidification rate (ECAR) of the media. This Application Brief describes the use of Agilent Seahorse XF technology to study the glycolytic engine in three recent publications.



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Gerriets; *et al.* (2015)², studied murine CD4⁺ T cell populations to determine whether metabolic programming affects the activation, differentiation, and function of CD4⁺ T cell subsets, specifically CD4⁺ effector T cells (Teffs), and regulatory T cells (Tregs). Teffs function in executing immunity and inflammation, while Tregs suppress Teffs to prevent excessive inflammation. Therefore, a balance is crucial to maintain immunological protection and prevent autoimmunity. Using Agilent Seahorse XF technology, the authors were able to characterize the metabolic phenotypes and substrate utilization capabilities between Teffs and Tregs, derived from *in vitro* differentiated murine CD4⁺CD25⁻ T cells. As illustrated in Figure 1, the CD4⁺ T cell subsets, Teff (Th1 and Th17), and Treg cells have distinct metabolic profiles. Upon further analysis, the authors noted a significant reduction in the glycolytic capacity and glycolytic reserve in Tregs, in comparison to Teffs (Figure 1B and 1C).

These data indicate that Teffs can engage in aerobic glycolysis, that is glycolysis in the presence of ample oxygen. In this study, the authors used an Agilent Seahorse XF24 Analyzer, in conjunction with the Agilent Seahorse XF Glycolysis Stress Test, and the Agilent Seahorse XF Cell Mito Stress Test to assess the metabolic phenotypes and glycolytic function of each murine CD4⁺CD25⁻ T cell subset. The Agilent Seahorse XF Glycolysis Stress Test was critical in illustrating the metabolic phenotype of Teffs, whereas the Agilent Seahorse XF Cell Mito Stress Test was crucial in determining the reliance of Tregs on oxidative phosphorylation. Moreover, their analyses show that the differences in metabolic phenotypes and fuel utilization capabilities can influence cell proliferation and function within the adaptive immune system. Studying the differences, including metabolic phenotypes and signatures, between these cell types may provide a viable therapeutic target to maintain balance or promote a desired response within the immune system.

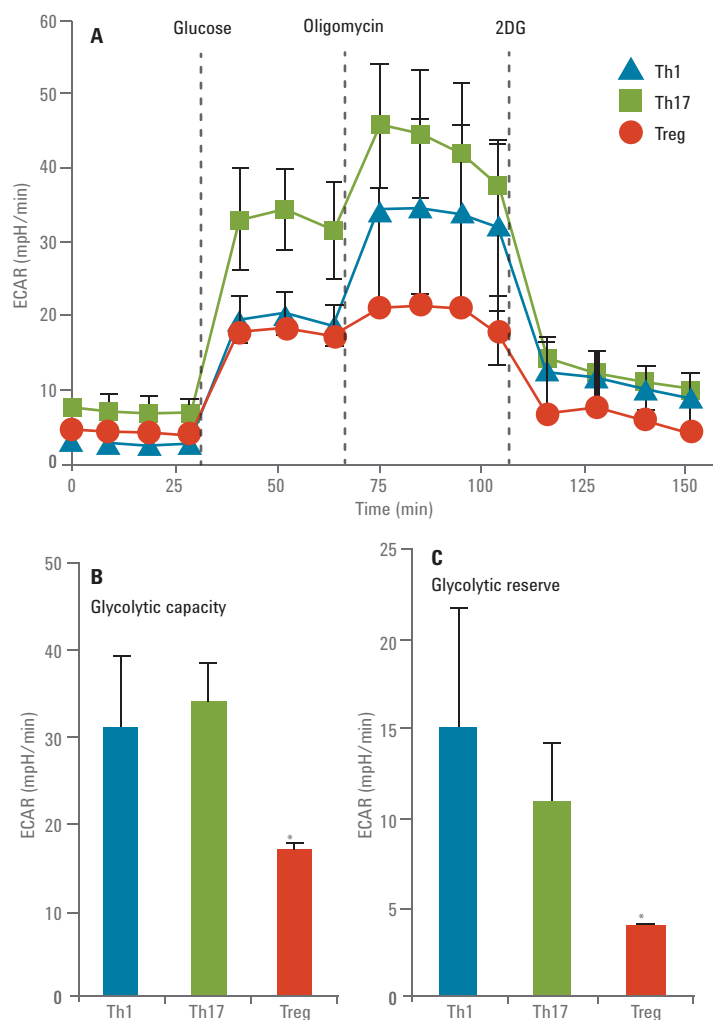


Figure 1. Teffs and Tregs have distinct metabolic phenotypes. A) CD4⁺CD25⁻ T cells were differentiated *in vitro*, passaged, and cultured with IL-2 to induce either TH1, Th17, or Tregs. ECAR was recorded following the addition of glucose, oligomycin, and 2DG. B and C) Calculations of glycolytic capacity (B), or glycolytic reserve (C).

Results and Discussion

Agilent Seahorse XF technology allows for the simultaneous measurement of the OCR and the ECAR in live, intact cells. By using the Agilent Seahorse XF Cell Mito Stress Test Kit, the Agilent Seahorse XF Glycolysis Stress Test Kit, and the Agilent Seahorse XFp Cell Energy Phenotype Test Kit, researchers can obtain functional metabolic data.

Similar to immune cells, neuronal cells also rely on cellular metabolism to maintain the high-energy demands of the cell. In cases of neurodegenerative

diseases, such as Parkinson's disease and amyotrophic lateral sclerosis (ALS), changes in metabolism play a key role in disease progression. In a study conducted by Allen; *et al.* (2014)³, Agilent Seahorse XF technology was used to examine fibroblasts isolated from ALS patients with the SOD1 genetic mutation, which has been identified as one of the most common genetic mutations in familial ALS (FALS) cases. The authors hypothesized that by using dermal fibroblasts from patients with the SOD1 mutation, not only could they recapitulate the functional defects observed in the

CNS of ALS patients, but also explore the interplay amongst the metabolic pathways that occurs during the disease state.

Using an Agilent Seahorse XF24 Analyzer, the authors assessed both mitochondrial respiration, measured by OCR, and the glycolytic activity, indicated by ECAR, in fibroblast cells taken from human skin biopsies in SOD1 ALS patients and age-matched controls. They observed that the rate of glycolytic activity was increased in SOD1 patient fibroblasts, without a parallel increase in glycolytic capacity. This observation suggested that the fibroblasts were operating at their maximal glycolytic capacity. The authors concluded that SOD1 patient-derived dermal fibroblast cells switched to glycolysis for ATP production for survival during metabolic stress. These data highlight the importance of discerning the energy pathways used by the cell for survival under normal and stress conditions.

Glycolysis is necessary for the production of energy in the form of ATP, as well as biological precursors used for cell growth and proliferation. In cancer cells, the conversion of glucose to lactate in the presence of ample oxygen is termed aerobic glycolysis, or the Warburg effect, and is a common characteristic often

referred to as a Hallmark of Cancer. The alteration in the metabolic pathway by these cells allows for their growth and proliferation, and underscores the need to research how metabolic alterations allow for tumor cell survival⁴.

Baek; *et al.* (2014)⁵, conducted a study evaluating lactate transporters in pancreatic ductal adenocarcinoma (PDA), a type of cancer that has a poor survival rate despite new therapies. The authors reasoned that as lactate production from glycolysis is readily adopted by cancer cells for survival, pinpointing a key lactate transporter, and disrupting its function, may inhibit pancreatic cancer cell viability and growth. The authors employed an Agilent Seahorse XF24 Analyzer and tested several human pancreatic adenocarcinoma lines, including PL45, Capan-2, and MIA PaCa-2. The Agilent Seahorse XF Glycolysis Stress Test was used to quantify the effect of MCT4, a key lactate transporter, on glucose metabolism. Using Agilent Seahorse XF technology, the authors determined that endogenous high-levels of MCT4 indicates a metabolic subtype, whereby cells are dependent on this specific transporter to maintain glycolytic activity. Upon further analysis, the authors were able to (1) conclude that MCT4 serves as a key marker and determinant of the metabolic state associated with

aggressive cancer, and (2) illustrate the metabolic pathways influenced by MCT4 that support cancer growth and proliferation.

Materials and Methods

Primary cultures of naive CD4⁺CD25⁻ T cells were obtained from 6–8 week old sex-matched C57BL/6J mice, and cultured on irradiated splenocytes in supplemented, RPMI 1640 media. To generate each subset, the following cytokines were added: Th1, 10 ng/mL IL-12, 10 µg/mL anti-IL-4, 1 µg/mL anti-IFN-γ; Th17, 20 ng/mL IL-6, 2.5 ng/mL TGF-β, 10 µg/mL anti-IGN-γ, Tregs, and 3 ng/mL TGF-β. Following 3 days of stimulation, cells were passaged and seeded with 20 ng/mL IL-2 for an additional 2 days prior to analysis.

XF Bioenergetic analysis

Metabolic analyses were performed using an Agilent Seahorse XF24 Extracellular Flux Analyzer, which enables the real-time, simultaneous rate measurements of oxygen consumption and extracellular acidification rate (OCR and ECAR, respectively), by creating a transient microchamber within each well of specialized cell culture microplates.

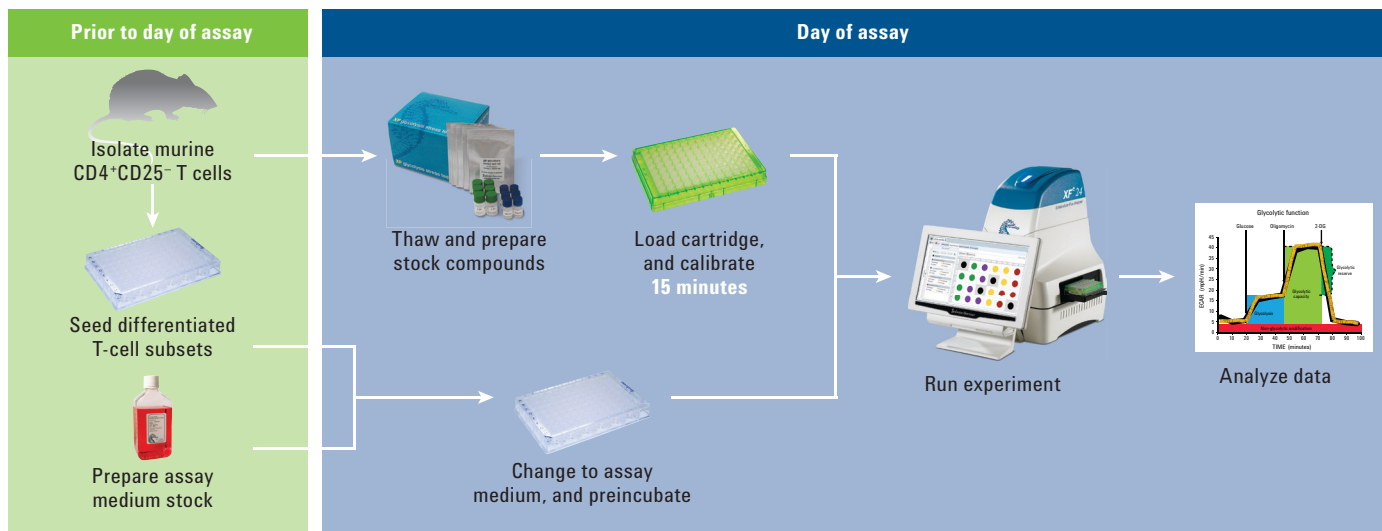


Figure 2. Flow chart of the XF assay.

As shown in Figure 2, T cells were seeded in Agilent Seahorse XF24 Cell Culture Microplates. Cell culture media was exchanged for Agilent Seahorse XF Base Medium with no added glucose or glutamine for the Agilent Seahorse XF Glycolysis Stress Test. Glycolytic capacity was calculated as the difference between ECAR following the injection of 1 μ m oligomycin, and the basal ECAR reading. Glycolytic reserve was calculated as the difference in ECAR between glucose and oligomycin injections. ECAR values were normalized to cell number.

References

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