Evaluating Changes in Cell Metabolism in Neuroimmune and Neuropsychiatric Disorders

Agilent Seahorse XF Technology Reveals Association Between Mitochondrial Function and Disease Diagnosis and Progression

Application Brief

Neurodegeneration, Immunology, Infectious Diseases

Introduction

The neuronal network, a complex series of intertwined pathways, molecules, and organelles, is particularly vulnerable to stress. Studies have shown that functional compromise within this intricate system may lead to disease. Within each neuronal cell, the mitochondria are responsible for generating ATP for energy, as well as governing cellular activities, including intracellular signaling, inducing antiviral response pathways, and cell survival. As neuronal cells are dependent on oxidative phosphorylation for energy, these cells are susceptible to mitochondrial dysfunction.

Several neurodegenerative diseases, including Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS), have been linked to mitochondrial dysfunction. However, the changes in cell metabolism associated with neuroimmune and neuropsychiatric disorders, such as encephalitis and lupus, have not been fully researched. This Application Note describes three publications that use Agilent Seahorse XF technology to quantify the changes in cell metabolism, which allows researchers to understand the pathophysiology of neuroimmune and neuropsychiatric disorders.

Systemic lupus erythematosus (SLE), the most common type of lupus, is an autoimmune syndrome that affects internal organs including the heart, joints, skin, lungs, kidneys, and nervous system. In a publication by Yin; *et al.* (2015)[1], the authors examined T cell metabolism in SLE patients. They hypothesized that lupus pathogenesis could be attenuated by T cell activation, and using metabolic modulators may reduce the severity, or reverse disease progression. Using XF technology, the authors compared the metabolic phenotypic differences in $CD4^+$ T cells derived from patients and healthy controls, the response to αCD3/αCD28 activation, and the effect of a potential therapy.

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As illustrated in Figure 1A, CD4+ T cells isolated from a murine SLE model and healthy, age-matched controls exhibited distinct metabolic profiles, such as increased ATP-linked respiration, maximum respiration, and spare respiratory capacity. The authors further confirmed that CD4+ T cells derived from SLE patients, as well as healthy controls, with and without anti-CD3 and anti-CD28 activation, similarly exhibited increased metabolism, specifically glycolytic activity (Figure 1B). As both metabolic pathways appear to be increased in the SLE mouse model and human SLE T cells, the authors then tested a combination therapy consisting of 2-Deoxy-D-glucose (2DG) and Metformin (Met). Figure 1C shows they observed a significant decrease in glycolytic activity in effector CD4+ T cells in the 2 months following treatment.

These data indicate that SLE is associated with a distinct metabolic phenotype, which is illustrated in CD4+ T cells. Furthermore, the authors demonstrated that a therapy involving both Met and 2DG can reverse the metabolic phenotype associated with SLE. The authors used Agilent Seahorse XFe96 and Agilent Seahorse XFe24 Extracellular Flux Analyzers (Seahorse Bioscience, a part of Agilent Technologies), and the Seahorse XF Cell Mito Stress Test to determine the metabolic phenotypes of cells derived from both disease and healthy controls. Moreover, they used real-time activation to observe changes in the T cell metabolic profile. This study underscores the exploration of T cell activation as a method of regulating SLE.

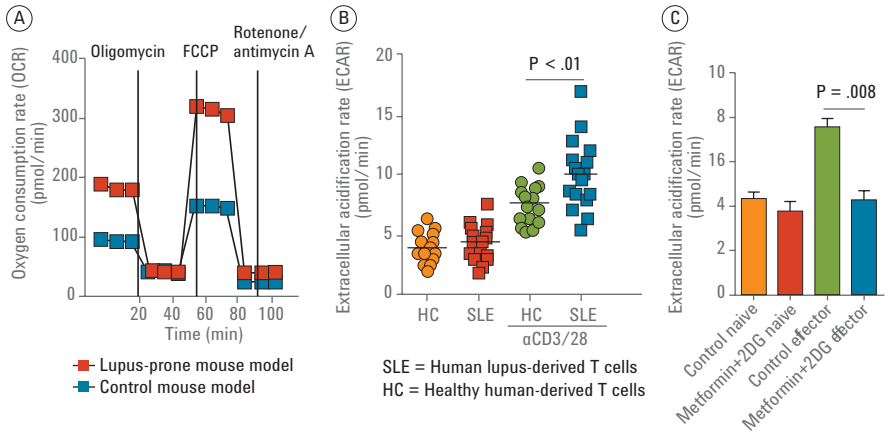

Figure 1. CD4+ T cells from lupus prone mice and humans exhibit increased mitochondrial respiration. A) Mitochondrial respiration of CD4+ T cells derived from control and lupus-prone mice was assessed using the XF Cell Mito Stress Test. B) Glycolytic activity of CD4+ T cells from human SLE patients, with and without anti-CD3/CD28 activation. C) Glycolytic activity was assessed in CD4+ T cells from the lupus-prone mouse model following a 2 month treatment with Metformin and 2DG.

Results and Discussion

There is ever-increasing evidence supporting the role of mitochondrial dysfunction in the pathophysiology of neuroimmune and neuropsychiatric disorders. Using Agilent Seahorse XF stress tests, kits, and reagents, researchers are exploring metabolic modulators as potential therapeutic treatments, and metabolic phenotypes as diagnostic or prognostic indicators.

Mitochondrial impairment has also been shown to play a significant role in viral pathogenesis, including encephalopathies, which predominantly affect the nervous system. During the viral life cycle, viral particles will often alter the host's cell metabolism to ensure efficient viral replication. As lipid metabolism provides a pathway to produce macromolecular precursors for viral production, determining the mechanisms involved in lipid oxidation is key to elucidating viral pathogenesis. In a study by Kao *et al.* (2015)[2], the authors researched the connection between fatty acid metabolism and Japanese viral encephalitis (JEV) pathogenesis. They hypothesized that lipid metabolism might contribute to JEV-triggered pathogenesis. Using a Seahorse XF24 Analyzer, the authors analyzed the response to a noncytotoxic dose of BSA conjugated palmitate (BSA-palmitate) in JEV-infected and healthy neuroblastoma cells. A decrease in mitochondrial respiration was observed indicating an ineffective usage of fatty acids, which may be due a blocked β-oxidation pathway. This relationship between fatty acid oxidation and JEV presents new findings in JEV pathogenesis and a potential therapeutic target.

With research into the connection between mitochondrial dysfunction, and neuroimmune and neuropsychiatric disorders intensifying, it is increasingly recognized that widespread mitochondrial dysfunction and increased oxidative stress, are linked to autism spectrum disorders (referred to as autism). In a study by Rose; *et al.* (2014)[3], a comparison was made on the effect of ethylmercury, a known environmental toxin, in lymphoblastoid cells isolated from individuals with autism and healthy controls. In comparison to the healthy controls, the authors observed a significant reduction in ATP-linked respiration, maximal respiratory capacity,

and reserve capacity in a subset of cells derived from the individuals with autism. The altered metabolic profile observed in the subset indicated a link between environmental influences, and adverse effects on neuronal development via mitochondrial dysfunction.

The authors used a Seahorse XF96 Analyzer and seeded lymphoblastoid cells from control and affected human donors. It was observed that a distinct metabolic profile emerged from a subset of lymphoblastoid cells isolated from individuals with autism. These observations highlight the susceptibility of some individuals to oxidative environmental toxins, such as ethylmercury.

Materials and Methods

Primary cultures of CD4⁺ T cells were either obtained from the spleens of female C57BL/6J mice, or isolated from the peripheral blood obtained from female human donors. For human SLE donors, each donor was treated with at least one nonbiologic treatment.

XF Bioenergetic analysis

Metabolic analyzes were performed using Seahorse XFe96 and XFe24 Analyzers, which enable 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 the specialized microplate.

Figure 2 shows that T cells were seeded in the appropriate Seahorse XF96 or XF24 Cell Culture Microplate. Cell culture media was exchanged to assay medium, consisting of nonbuffered RPMI medium supplemented with 2.5 µm dextrose, 2 mM glutamine, and 1 µm sodium pyruvate. The Seahorse XF Cell Mito Stress Test was then used to determine mitochondrial respiration following the sequential injections of oligomycin, FCCP, and a combination of rotenone and antimycin A.

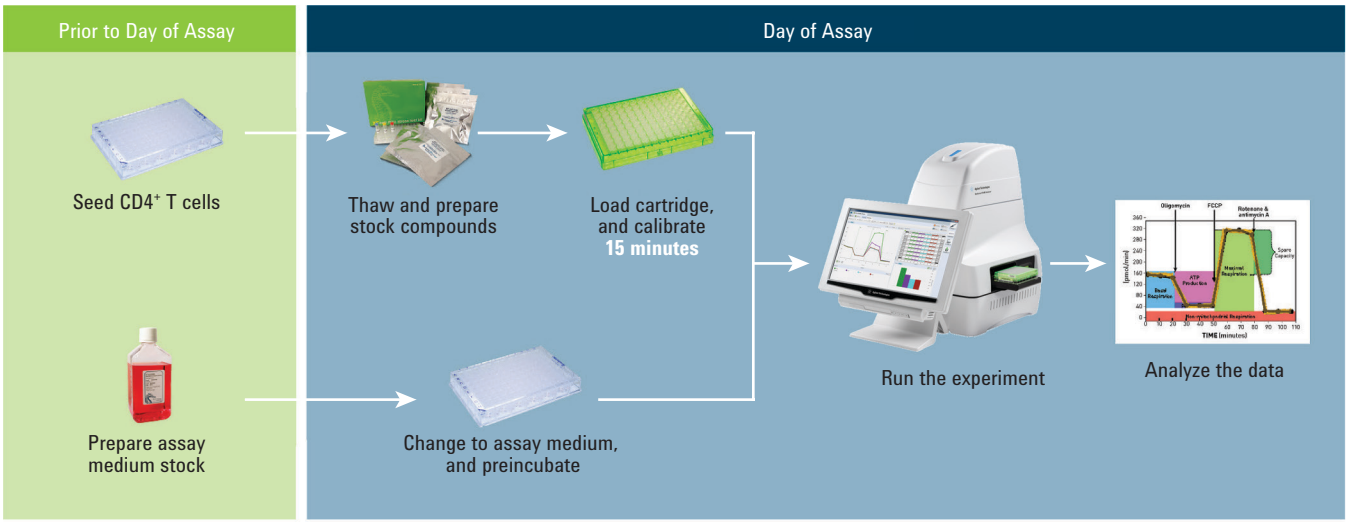

Figure 2. Flow chart of the XF Assay.

References

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www.agilent.com/chem/discoverXF

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