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"The effect of Chronic Alcohol on CD4+ T cell Metabolic Programming"

Alcohol use is common in people living with HIV and is associated with poor disease outcomes. Previous studies by the LSU Comprehensive Alcohol Research Center showed that chronic-binge alcohol administration to simian immunodeficiency virus (SIV) infected rhesus macaques increased CD4 T cell proliferation and activation in the intestinal tract. Further, after SIV infection, plasma viral loads were higher than in non CBA SIV-infected macaques. However, the physiological mechanisms associated with T cell proliferation are incompletely understood. During activation, CD4 T cells, the primary target of HIV, undergo a metabolic switch from oxidative phosphorylation to glycolysis to maintain adequate energy production. This metabolic switch is essential to efficiently differentiate into effector T cells. Naïve T cells maintain energy homeostasis using oxidative phosphorylation due to its high efficiency, making up to 15 times as much ATP as glycolysis. However, when CD4 T cells are activated, the switch to glycolysis is beneficial because of the increased rate of ATP production, which is used in cellular processes required for T cell proliferation and differentiation. We propose a conceptual model in which ethanol exposure dysregulates expression of regulators of the metabolic switch from oxidative phosphorylation to glycolysis, impairing normal CD4+ differentiation. Specifically, we hypothesized that ethanol exposure alters peripheral blood mononuclear cell (PBMC) expression of genes regulating the metabolic switch from oxidative phosphorylation to glycolysis.

Methods: Human PBMCs were cultured *in vitro* with 0 mM, 25 mM, or 50 mM ethanol for 24 hours (acute exposure) or 7 days (chronic exposure) (n= 4-6/group). After the exposure time, PBMCs are stimulated with phorbol myristate acetate (PMA) and ionomycin for 4 hours followed by RNA isolation using the RNeasy Mini Kit. cDNA was generated by reverse transcription using the iScript cDNA Synthesis kit. Real time PCR was performed on a Bio-Rad Thermal Cycler using SSO Advanced Universal SYBR Green supermix for the following genes: Raptor, Rictor, B-cell lymphoma 6 (BCL-6), activated protein kinase (AMPK), Pyruvate Dehydrogenase, Hexokinase, peroxisome proliferator-activated receptor gamma coactivator (PGC)1-alpha, and PGC1-beta. Results were analyzed using Excel. An alpha error less than 0.05 was considered statistically significant.

Results & Discussion: Preliminary analysis of cells incubated for 24-hour in 50 mM ethanol and controls showed that the PMA and Ionomycin stimulated cells incubated with 50 mM ethanol, show a trend for increased expression of Raptor, a component of the mammalian target of rapamycin (mTOR) pathway, and a decreased expression of BCL-6, a marker for follicular helper cells. No significant differences were seen in expression of other genes. Raptor expression trended higher in the ethanol-treated cells as compared to the control groups, suggesting an increase in mTORC1 expression and, therefore, glycolysis. BCL-6 expression decreased (p=NS) in ethanol versus control in the stimulated groups, suggesting that follicular helper cell differentiation is impaired or reduced in the presence of acute ethanol exposure. Additional experiments are in progress.