Effect of Acute Alcohol on Peripheral Blood Mononuclear Cell Metabolism

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Introduction

- Alcohol impairs the host defense immune response to infection by viruses, like HIV, and by a variety of bacteria.
- Published work has shown that acute exposure to ethanol decreases immune cell activation and proliferation both in preclinical animal models and *in vitro* cell culture.
- Recently, the importance of metabolic control of immune activation and differentiation has been described.
- Optimal proliferation and differentiation requires a metabolic switch from the efficient, but slow, production of ATP by oxidative phosphorylation to more rapid ATP generation by glycolysis (at the expense of efficiency).
- However, the mechanistic role of this metabolic switch in ethanol's effects on immune cell activation, proliferation, and differentiation is unknown.

Materials and Methods

- Cell Culture: PBMCs were isolated from healthy donors by density gradient centrifugation (Ficol-Paque) supplied by the Blood Bank from healthy donors. Cells were cultured in RPMI media with 10% FBS, 1% L-glutamine, 1% penicillin/streptomycin, and 3 pg/ul recombinant human IL-2.
- *In-vitro* Treatments: Acute ethanol exposure was performed by incubation in media with 25mM or 50mM of ethanol for 24 hours. After ethanol exposure, cells were stimulated with 50 ng/mL phorbol myristate acetate (PMA) and 1 ug/mL Ionomycin for 4 hours followed by RNA isolation.
- RT-qPCR: cDNA was then generated for each sample. Expression was measured using RT-qPCR on a Bio-Rad CFX96 Thermo Cycler using SSO Advanced Universal SYBR Green supermix for the following metabolism-related genes: Raptor, Rictor, and BCL-6. Expression was normalized to the housekeeping gene RPS13.
- Data Analysis: qPCR data was normalized to the untreated for each donor. All data was analyzed using Excel & GraphPad Prism 8.0. Statistical testing was performed using a 2-way ANOVA with Tukey's multiple comparison test. An alpha error < 0.05 was considered statistically significant.

PBMC expression of metabolic genes after acute 50 mM ethanol exposure.

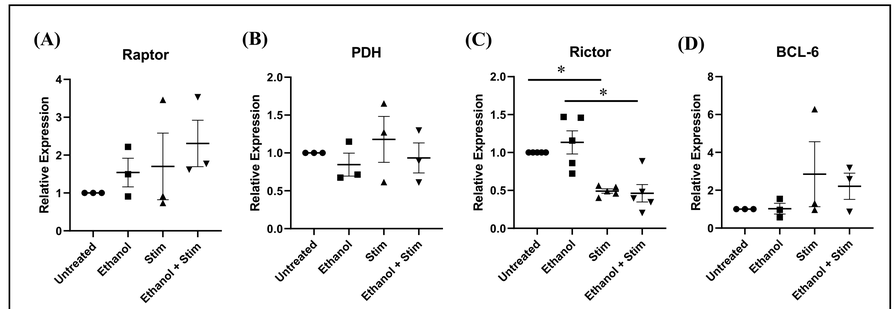
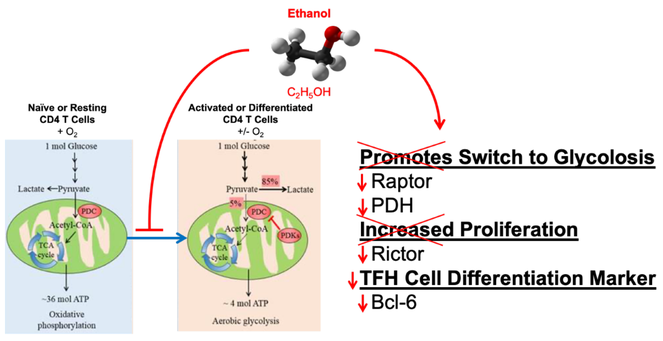


Figure 2. 50mM Ethanol effects on metabolic gene expression. (A) Raptor expression increased in the ethanol + stim group as compared to the stim group, though it was not statistically significant. Expression also increased in the unstimulated ethanol group as compared to the untreated group. (B) PDH expression was unchanged. (C) Rictor expression decreased significantly in both stimulated groups as compared to the unstimulated groups. (D) BCL-6 expression decreased when in the presence of acute ethanol after stimulation. N=4-6/group. P< 0.0001 for Rictor. Other results were not statistically significant.

Conceptual Model



Hypothesis

Ethanol exposure decreases peripheral blood mononuclear cell (PBMC) expression of genes mediating the metabolic switch from oxidative phosphorylation to glycolysis.

PBMC expression of metabolic genes after acute 25 mM ethanol exposure.

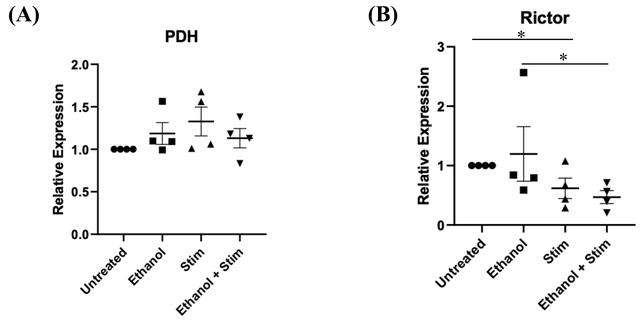


Figure 1. 25mM Ethanol effects on metabolic gene expression. (A) There were no changes for PDH expression. (B) Rictor expression decreased significantly in both stimulated groups as compared to unstimulated groups. N=5/group. P=0.0475 for Rictor. PDH was not statistically significant.

Conclusions

- We did not observe an ethanol effect.
- We saw a decrease in Rictor related to stimulation however there was no ethanol effect.
- There was no statistically significant difference in other genes based upon either stimulation or ethanol.

Future Experiments

- Continue PCR to determine gene expression for the alternate concentrations (25mM & 50mM) and timepoints (7 day).
- Assess differentiation of CD4 by flow cytometry after ethanol exposure

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