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"Combination of In-Vitro Stimulation and 50 mM Ethanol Treatment on Healthy Blood Bank PBMCs increases Activated Senescent CD8 T-cells"

Background: Cellular senescence refers to a phenotypic state where a cell can no longer divide due to telomere attrition or DNA damage. Senescent T cells, while incapable of dividing and hyporesponsive to antigens, produce non-specific proinflammatory mediators that are collectively known as the senescence-associated secretory phenotype (SASP). As humans age, senescent cells accumulate and contribute to chronic inflammation, known as "inflamm-aging". In addition, HIV and alcohol are known to accentuate and accelerate various aspects of biological aging. In people with HIV (PWH) with alcohol use disorder (AUD), there are higher levels of activated senescent immune cells and chronic persistent inflammation. Previous preliminary data show that in PWH and alcohol misuse, there is a positive association in CD8 T cell senescence, intestinal leak, and dysbiosis.

Hypothesis: In-vitro stimulation and exposure to 50 mM ethanol (EtOH) will contribute to an increase in activated senescent CD8 T cells.

Methods: Healthy blood bank donor-derived peripheral blood mononuclear cells (PBMCs) were thawed and left overnight to recover in a 37.0° C humidified incubator with 5% CO. After 24 hours, they were washed and incubated in RPMI 1640 with and without 50 mM EtOH and anti-CD3 antibody-coated wells. After 1- and 24-hour incubation, a staining panel is added to label CD3, CD38, active caspases 1 and 3, CD-279, CD14, CD4, CD20, CD28, CD8, Ki67, and Live/Dead. Three markers were used to identify activated senescent cells: CD38 was used to identify cell proliferation, CD8 to identify all CD8 cells, and loss of CD28 as a senescent marker. PBMCs are analyzed using a BD LSR II and DIVA version 8.0.1 for identification of markers.

Results: With Trypan blue cell counting, there was a decrease in PBMC live/dead ratio from the 0-hour mark to the 24-hour mark within all treatment groups. With flow analysis, the percentage of CD3+ live cells were lower at the 24-hour time point compared to other time points and ethanol further decreased the number of live cells. Assessment of CD8 T-cells showed a decrease in the percentage of CD8+ T cells exposed to ethanol and ethanol with stimulation at the 24-hour time point. There were higher CD8+CD38+T cells and CD8+CD28-CD38+ T cells in the ethanol and ethanol stimulation groups at 24 hours. All data will be analyzed for statistical significance via repeated measures 2-way ANOVA.

Conclusions: Our results suggest that 24-hour EtOH treatments decreases live CD3+ cells. EtOH increased the population of activated senescent CD8 T cell at 24 hours. Future analysis will be to explore cell death pathways occurring in these inflammatory immune cells in the context of stimulation and EtOH.

Special Instructions: The abstract is a summary of the project. Do not to exceed one page. Do not change margins, font style or font sizes on this page. **Use this format only- do not modify!!!**

Body of Abstract: Left Justified, 11 point Arial font, single-spaced, double space between paragraphs.