# Understanding Effects of *In Vitro* Stimulation and 50 mM EtOH on Blood Bank PBMCs

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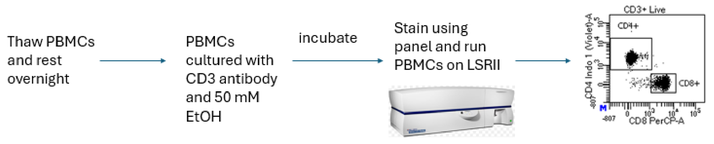
## Background

- Cellular senescence = phenotypic state whereby a cell can no longer divide due to telomere attrition or DNA damage
- Senescent cells accumulate and contribute to chronic inflammation during the aging process, contributing to “inflamm-aging”.
- In people with HIV (PWH) with alcohol use disorder (AUD), there are higher levels of activated senescent CD8+ T 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 T cell receptor (TCR) stimulation in the presence of 50 mM ethanol (EtOH) will lead to a greater increase in activated senescent CD8 T cells than with TCR stimulation alone.

## Methods




- Figure 1: Experimental workflow for PBMC phenotypic analysis
- Experimental groups: 1. Untreated (U), 2. 50 mM EtOH (E) only, 3. anti-CD3 antibody stimulation only (US), & 4. CD3 antibody/ 50 mM EtOH (ES)
- Cryopreserved blood bank donor-derived peripheral blood mononuclear cells (PBMCs) were thawed & cultured overnight to recover in a 37.0°C humidified incubator with 5% $CO_2$.
- PBMCs are then incubated in RPMI 1640 with and without 50 mM EtOH and anti-CD3 antibody-coated wells for multiple time points (0, 1.5, & 24 hrs).
- Samples were stained for viability and active caspase 1, followed by immunostaining for CD3, CD4, CD8, CD14, CD20, CD28, CD38, CD279, active caspase 3, and Ki67.
- Activated Senescent CD8+ T cells (Act Sen) defined as CD8+CD28-CD38+

## Ethanol Effects on Viability

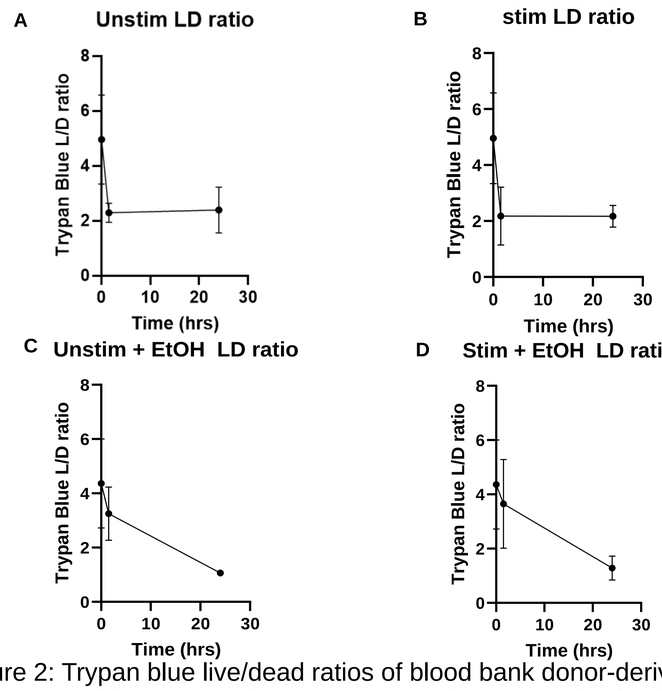



Figure 2: Trypan blue live/dead ratios of blood bank donor-derived PBMCs exposed to different stimuli over 24 hours. **A) untreated. B) Stimulation – anti-CD3 C) EtOH only. D) EtOH and stimulation – anti-CD3**

## CD8 T Cell Activation

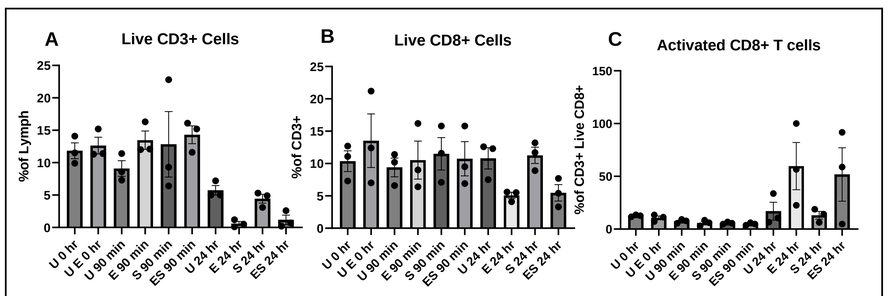



**Figure 3:** Frequencies of live and activated CD3+ T-cells within blood bank PBMCs exposed to stimuli over 24 hours. **A)** CD3+ live cells. **B)** CD3+CD8+ live cells. **C)** CD3+CD8+CD38+ live cells. **U - untreated, E - 50mM EtOH, S - anti-CD3, ES – 50 mM EtOH + anti-CD3**

## EtOH Increased the Ratio of CD8+CD28+/CD8-CD28-

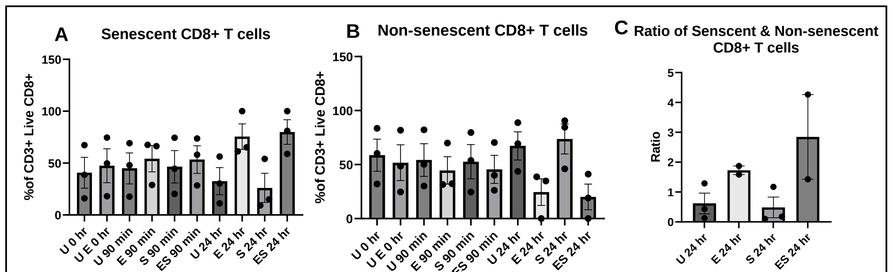



**Figure 4:** Percentages and ratios of CD8+CD28- T cells and CD8+CD28+ T cells within healthy blood bank PBMCs after 24 hours in culture **A)** Percentage of live CD8+CD28- T-cells over 24 hours **B)** Percentage of live CD8+CD28- T cells over 24 hours. **C)** Ratio of live CD8+CD28- T cells to live CD8+CD28+ T cells. **U - untreated, E - 50mM EtOH, S - anti-CD3, ES – 50 mM EtOH + anti-CD3**

## EtOH -> Activated senescence

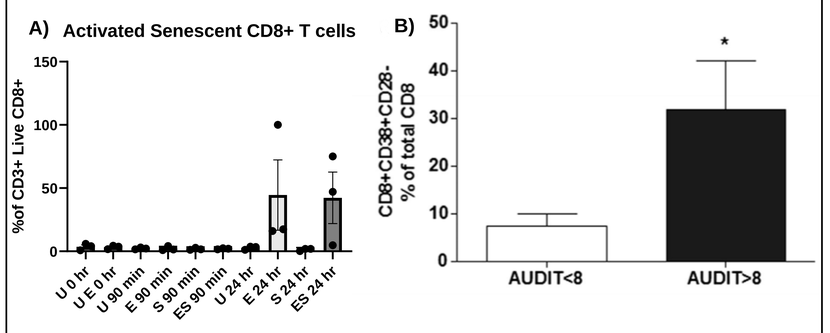



Figure 5: **A)** Percentage of activated immunosenescent CD3+CD8+CD28-CD38+ T cells derived from blood bank PBMCs after 24 hours**. B)** Percentage of activated immunosenescent (CD3+CD4-CD8+CD38+CD28-) T-cell human PBMCs isolated from PWH with either low or high AUDIT score (Katz, et al. 2015). **U - control, UE - 50mM EtOH, S - anti-CD3, ES - 50 mM EtOH + anti-CD3**

## Summary and Conclusions

- Trypan blue cell count results showed a decrease the live/dead ratio from 0 hr to 24 hr within all treatment groups.
- FACS analysis showed the percentage of total live CD3+ cells and CD8+ T cells were lower at the 24 hr time point compared to other time points, and ethanol further decreased the frequency of live cells.
- The CD8+CD28-/CD8+CD28+ T cell ratio was highest at 24 hours.
- There were higher amounts of CD8+CD38+T cells and CD8+CD28-CD38+ T cells (Act Sen) in the EtOH and EtOH + stimulation groups at 24 hours.
- ***In vitro* 50 mM EtOH treatment and TCR stimulation (anti-CD3) increases activated senescent CD8+ T cells in PBMCs, similar to our *in vivo* observations in both the chronic binge alcohol (CBA)-administered SIV-infected rhesus macaque and in PWH and alcohol misuse.**
- **Our data suggests that this model could be used to mechanistically explore pathways affected by alcohol and inflammatory mediators that promote senescence**

## References

Katz, P. S., Siggins, R. W., Porretta, C., Armstrong, M. L., Zea, A. H., Mercante, D. E., Parsons, C., Veazey, R. S., Bagby, G. J., Nelson, S., Molina, P. E., & Welsh, D. A. (2015). Chronic alcohol increases CD8+ T-cell immunosenescence in simian immunodeficiency virus-infected rhesus macaques. *Alcohol*, *49*(8), 759–765. https://doi.org/10.1016/j.alcohol.2015.09.003

**Funding:** P60AA009803