CHRONIC BINGE ALCOHOL INCREASES INFLAMMATORY DEATH PATHWAY ACTIVATION IN SENESCENT CD8+ T CELLS

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Background

- With the availability of highly effective ART, HIV has become a chronic condition characterized as a premature aging.
- Aging and geriatric comorbidities are associated with the onset of cellular senescence, which is defined as permanent proliferative arrest.[1]
- The immunopathy associated with HIV parallels aging-related immunosenescence, represented as diminished immune defense against infection associated with excessive, non-specific inflammation (inflammaging).[2,3,4]

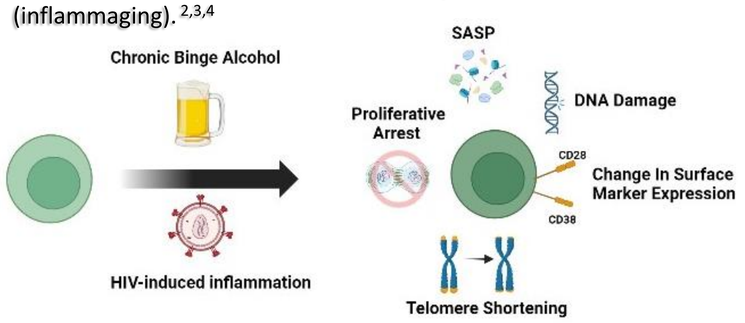

Figure 1. Effects of alcohol and HIV on cellular senescence. HIV promotes persistent inflammation which parallels immunosenescence.[2] Long term exposure to ethanol leads to phenotypic changes in T cells similar to immunosenescence.[3]

- Persons With HIV (PWH) have a 2–3-fold higher prevalence of alcohol use disorder (AUD) than individuals in the general population.[5,6,7,8]
- At-risk alcohol use promotes a pro-inflammatory environment. Chronic Binge Alcohol (CBA) and alcohol use augment immune activation and immunosenescence in SIV-infected male macaques and PWH.[10]
- Senescent cells are known to be resistant to apoptosis (marked by Caspase 3 activation which contributes to an accumulation of senescent cells with aging and with HIV.

Hypothesis

Senescent CD8+ T cells in CBA-administered female rhesus macaques are susceptible to alternative, proinflammatory cell death pathways, including pyroptosis (marked by Caspase 1 activation) and necroptosis. Chronic binge alcohol consumption increases these cell death mechanism in senescent cells.

Methods

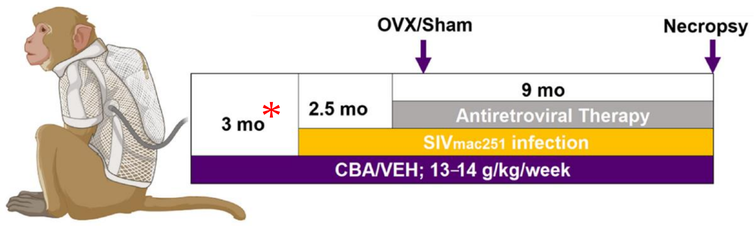

Figure 2. Macaque study design. Samples were taken after 3 months of CBA (figure from published model by McTernan et al).

Experimental groups: VEH only, CBA only, VEH/SIV, CBA/SIV
Alcohol Administration: Macaques were administered alcohol intragastrically at a concentration of 30% (wt/vol) in water (30 min infusion; 5 days/wk; 12–15 g/kg/wk). Peak plasma alcohol concentrations averaged 50–60mM(~230–280 mg%) 2 h after alcohol initiation.
SIV infection: After 3 months of CBA/VEH administration (pre-simian immunodeficiency virus (SIV)), all animals were infected intravaginally with SIVmac251

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Staining Protocol

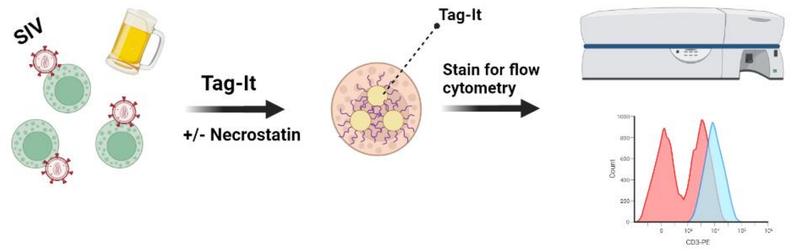

Figure 3. Staining protocol for rhesus macaque peripheral blood mononuclear cells (PBMCs; image designed in Biorender).
Cryopreserved PBMCs were thawed and incubated in Thawing media composed of FBS 10%, Glutamine, Pen Strep. After thawing, cells were stained with Tag-It, treated with Necrostatin-1, and rested overnight. The next morning (18 hours later), cells were washed and stained with the following antibody panel: Viability Dye- eFluor780, CD3-BV605, CD4-BUV395, CD8-PerCP, CD28-PE-Cy7, CD279-BV510, CD38-AF700, CD14-PE, CD20-BV570, Caspase-1- FITC,Caspase-3-AF647, and Ki67-PEDazzle594. Cells were analyzed on the BDBiosciences LSRII and FACSDIVA version 8.0.1.

Gating Strategy

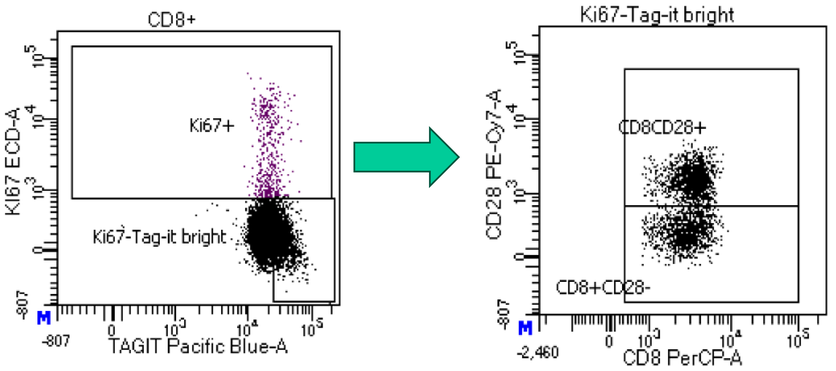

Figure 4. Cells were analyzed one the BDBiosciences LSRII and FACSDIVA version 8.0.1.
Phenotypes for CD8+ T cell immunosenescence: CD3+CD4-CD8+CD28-Ki67-,Tag-It Bright

CBA Increases Frequency of Senescent CD8+ T-cells

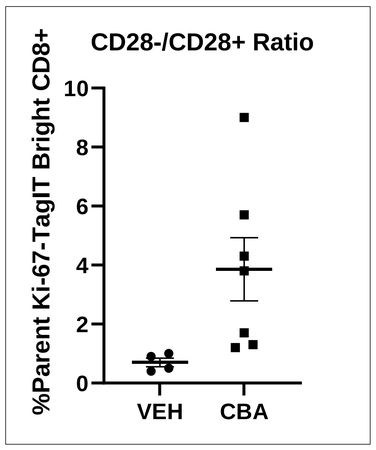

Figure 5. Effect of CBA on senescence-enriched cell frequency within the population of Ki67- TagIt Bright CD8+ and CD28- cells. CBA animals appear to have a higher CD28-/CD28+ cell ratio compared with VEH animals.

Acknowledgments

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CBA Shifts Senescent CD8+ T-cells to Pyroptosis

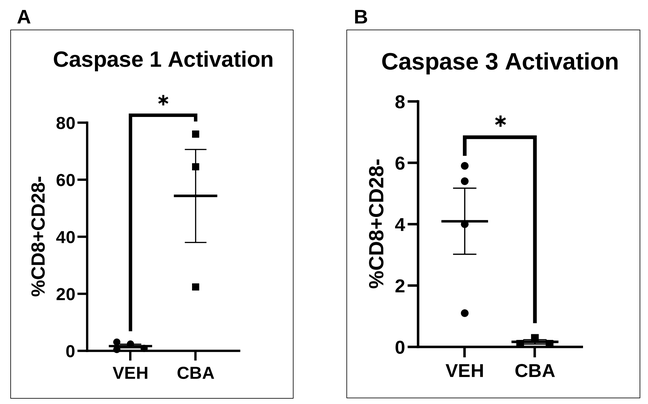

Figure 4. Senescent CD8+ T cells within CBA animals had higher activation of inflammatory cell death.
A. CBA animals had higher Caspase 1 (pyroptosis) activation compared to VEH animals within the senescent CD8+ T cell population.
B. CBA animals had less Caspase 3 (apoptosis) activation compared to VEH animals within the senescent CD8+ T cell population.

Summary & Conclusion

- We have developed a panel to assess senescent CD8 T-cells within PBMCs.
- Consistent with prior results, we observed an increase (non-significant, likely due to limited sample size) in senescent CD8+CD28- cells in CBA-administered animals.
- Alcohol consumption decreased Cas 3 (apoptotic) activation and increased in Cas 1 activation (indicating pyroptosis) within the senescent CD8+ T cell population from CBA animals consistent with a shift from regulated, non-inflammatory cell death to pro-inflammatory cell death

Future Directions:

- Work is ongoing to assess other major timepoints from the CARC rhesus macaque model to determine whether these changes in senescent cells lead to changes in viral progression.

Conclusion:

Senescent cells contribute to the increased inflammation associated with geriatric comorbidities. The observed increase in the senescent cells undergoing pyroptosis could contribute to a pro-inflammatory milieu in CBA-administered animals. If confirmed, this represents a paradigm shift in our understanding of the mechanisms central to Inflammaging.

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