Impaired AICD in Senescent CD8 T-Cells: Cell Death Pathway Selectivity & the Effects of Alcohol

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Introduction

- Alcohol Use Disorder (AUD) is prevalent in People Living with HIV (PLWH)
- Alcohol & HIV may accelerate the onset of cellular senescence; Pro-inflammatory senescent cells are associated with unhealthy aging
- Activation-induced cell death (AICD) is observed to be impaired in senescent cells, & the mechanism of this impairment of cell death unknown.

Hypothesis

Senescent CD8 T-cells are protected from apoptosis, but vulnerable to necroptosis and pyroptosis, and alcohol will increase cell death through non-apoptotic pathways.

Study Design & Methods

We stimulated PBMCs from healthy human donors with T-cell activation ligands (anti-CD3 and anti-CD28) and then treated these cultures with reagents specific to a cell death pathway.

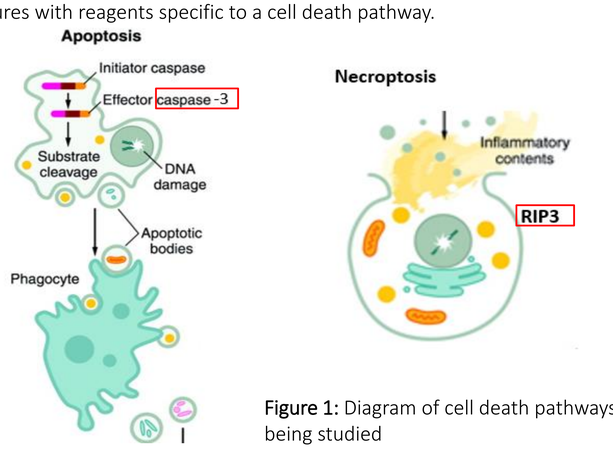


Figure 1: Diagram of cell death pathways being studied

Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect Immun. 2005 Apr;73(4):1907-16.

- Apoptosis: Caspase 3
  - FasL
  - Cisplatin
- Pyroptosis: Caspase 1
  - N-3-oxo-dodecyl-L-homoserine Lactone
- Necroptosis: RIP3
  - TNFα (+actinomycin D)

Table 1: Treatment Conditions	Control	Low Dose	High Dose	Time 1	Time 2
TNF (+ actinomycin D)	Veh	5 ng/mL (0.2ug/mL)	10 ng/mL (0.2ug/mL)	6 hours	24 hours
Lactone	Veh	200 µM	500 µM	6 hours	24 hours
Cisplatin	Veh	250 µM	500 µM	6 hours	24 hours
FasL	Veh	400 ng/mL	800 ng/mL	6 hours	24 hours

Results

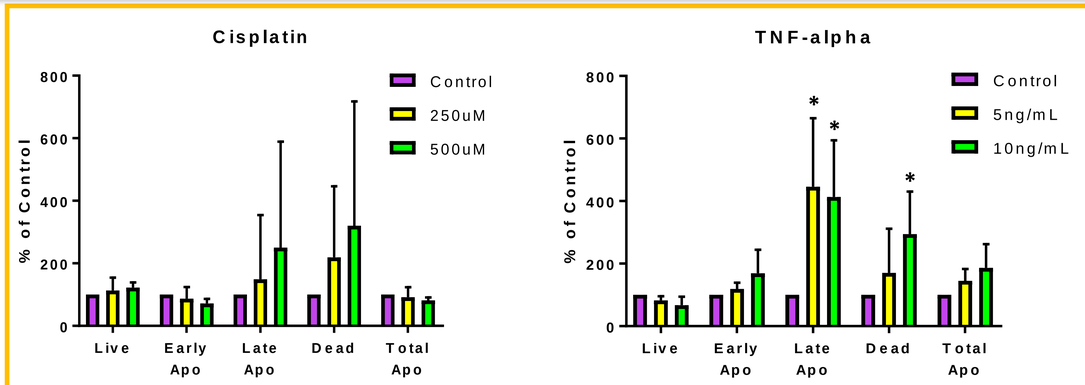


Figure 2: Effect of TNFα (A) and Cisplatin (B) on Percent Gated of Live, Apoptotic, and Dead cells. Values presented as percent relative to untreated 6-hour control. Statistics were analyzed using Tukey Honest Significant Difference.

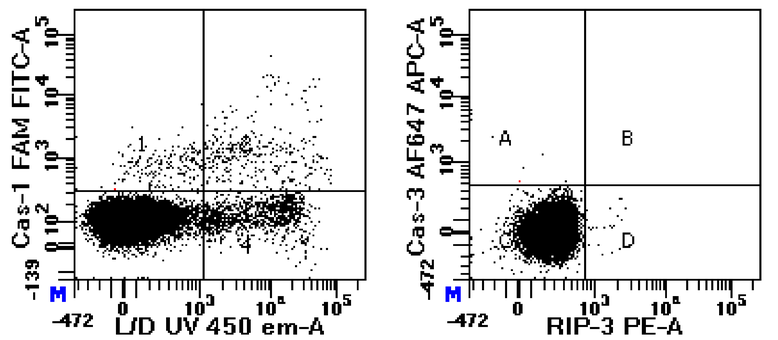
*- Significant difference ($p < 0.05$) was determined when compared to control group.

Conclusion & Discussion

We identified the optimal treatment with TNFα to be 10 ng/mL (with 0.2 ug/mL actinomycin D) for 6 hours and cisplatin to be 250 µM for 6 hours. We observed no effect of FasL and 3-oxo-C12-HSL treatments for the doses and time courses we tested. Our current data allows us to achieve an optimal amount of apoptosis and necroptosis so that we may study populations of senescent cells in each.

Future Directions

- Analyze specific cell death pathways proteins and senescence phenotypic markers in each treatment to in order to determine if senescent cells are differentially protected from each cell death pathway.
- Repeat these experiments in the presence of 50 mM alcohol to understand the direct effects of alcohol use on cell death pathways in senescent in CD8 T-cells.



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