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

Background: Alcohol Use Disorder (AUD) is prevalent in People Living with HIV (PLWH). Chronic alcohol use and HIV infections are both stressors that can accelerate the onset of cellular senescence, a pro-inflammatory phenotype associated with unhealthy ageing and contributor to the phenomenon known as “inflamm-aging.” Furthermore, it has been observed that in senescent cells, there is an impairment of Activation-Induced Cell Death (AICD). The mechanism of impairment of AICD in senescent cells remains unknown. The goal of our research is to investigate by which pathway of cell death (apoptosis, pyroptosis, necroptosis) senescent cells are susceptible by analyzing markers of senescence (CD54, KLRG1, and SA-β-gal) in CD8 T-cells that are induced to undergo a certain type of cell death. We hypothesize that senescent CD8 T-cells are protected from apoptosis but vulnerable to necroptosis and pyroptosis. **Methods:** To investigate these multiple death pathways and the ability of senescent CD8 T-cells to undergo them, we designed an experiment in which we stimulate PBMCs from healthy human donors with T-cell activation ligands anti-CD3 and anti-CD28 and then treat these cells with reagents specific to each pathway. We have identified FasL and Cisplatin as physiological and chemical inducers of apoptosis, respectively. We used TNFα, in conjunction with Actinomycin D, as an inducer of apoptosis and necroptosis. We induced the pyroptotic death pathway with N-3-oxo-dodecyl-L-homoserine Lactone (3-oxo-C12-HSL). After determining an appropriate dose and time course for treatment with each reagent to achieve approximately 50% cell death using Luminex Muse flow cytometry, we will utilize these treatment conditions on PBMCs for analysis of markers of senescence in order to elucidate the differential protection of senescent cells in cell death pathways. **Results:** Our data indicates that a 6-hour treatment of TNFα+actinomycin at 10 ng/mL (with .2 ug/mL actinomycin D) is optimal for achieving the amount of cell death for us to further study senescence in apoptotic and necroptotic pathways. We have also identified a 6-hour treatment of 250 μM cisplatin to allow us to further study senescence in apoptosis. We observed no effect of FasL and 3-oxo-C12-HSL treatments for the doses and time courses we tested. **Discussion:** Our current data allows us to achieve an optimal amount of cell death in each pathway so that we may study populations of senescent cells in each. **Future Directions:** Having determined the optimal doses and times of treatments to induce each death pathway to be studied, we will again stimulate these pathways for a more in-depth analysis examining specific cell death pathways proteins and senescence phenotypic markers. We will use the positive markers CD57, KLRG1, and SA-β-gal, as well as markers that are down-regulated in senescence (CD27, CD28, and TERT) to identify senescent cells in each culture. This will allow us to determine if senescent cells are differentially protected from each cell death pathway. We will 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.