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"Interferon-gamma expression increases in senescent immune cells following stimulation of peripheral blood mononuclear cells"

BACKGROUND: Biological aging is associated with decline in physical function and an increased risk of disease. Hallmarks of biological aging are cellular and molecular changes that contribute to the aging process, including cellular senescence. Senescent cells can no longer divide, are resistant to apoptosis, and secrete a senescent-associated secretory phenotype (SASP). Senescence can lead to chronic systemic inflammation and contribute to age-associated comorbidities. Various lifestyle factors including alcohol may accelerate biological aging. This objective of the study was to develop a flow cytometry panel to identify senescent and associated cytokine expression in peripheral blood mononuclear cells. Our long term goal is to identify whether alcohol increases markers of cellular senescence in older adults.

OBJECTIVE: This preliminary study aims to develop and apply a flow cytometry–based assay to identify senescent immune cell and the associated IFN-gamma expression.

METHODS: The samples used for this study were peripheral blood mononuclear cells (PBMCs) from blood bank donors. The antibodies used for staining were Tag-it, CD8, CD45, CD38, CD14, CD20, VIAB, CD4, CD28, CD3, Ki-67, IFN-gamma, IL-18, and Beta-galactosidase. The controls included an unstained sample, single-stained samples, and Fluorescence Minus One controls (FMOs). PBMC samples from 3 blood bank donors were first thawed and counted, incubated for 24 hours at 37C 5% CO2 and 100% relative humidity. The following day, cells were counted, stained with Tag-it, stimulated using 1ug/100mL PMA and 1ug/mL Ionomycin, and incubated for another 24 hours. The next day, Golgi block was added to each sample for 4 hours, then they were stained with the remaining extracellular stains, fixed, stained with the intracellular stains, and incubated for 12 hours. The Cytek Northern light flow cytometer was used for flow cytometry analysis.

RESULTS: Stimulation with PMA and ionomycin increases CD38 expression (activation marker) in both CD4 and CD8 cells. CD38 expression in CD8 cells was 35% and that of CD4 cells was 68%. Beta-gal expression (MFI) was $1.25x10^7$ in CD8 cells and $1.0x10^7$ in CD4 cells. Stimulation has no effect on Beta-gal expression in CD4 and CD8 cells. Stimulation increases IFN-gamma expression in total CD4 cells, and senescent CD4+CD28- cells (17%) have higher frequency of IFN-gamma than non-senescent CD4 cells (3%). Stimulation also increases IFN-gamma expression in CD8 cells (33%). Stimulation increases IFN-gamma expression in senescent CD8+CD28- cells more than non-senescent CD8 cells (38%). Stimulation increases IFN-gamma expression in activated senescent CD8+CD28-CD38+ cells (35%). Comparing non-senescent CD4 and CD8 cells, there was increased IFN-gamma expression in non-senescent CD8 cells than non-senescent CD4 cells.

CONCLUSION: This study developed a flow cytometry assay to identify senescent T cell populations and assess IFN-gamma expression. Results show that stimulation increases immune activation and pro-inflammatory cytokine expression, especially in senescent CD8 and CD4 cells. These findings suggest that senescent immune cells have a higher frequency of inflammatory cytokine production, which may contribute to age-related inflammation.