Interferon-gamma Expression Increases in Senescent Immune Cells Following Stimulation of Peripheral Blood Mononuclear Cells

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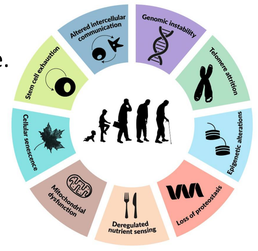
Introduction



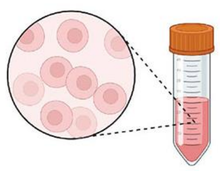
Biological aging is associated with decline in physical function and increased risk of disease.
Hallmarks of aging are cellular and molecular changes that contribute to aging
Senescent cells do not divide, are resistant to apoptosis, and secrete a senescence-associated secretory phenotype (SASP).
Senescence can lead to systemic inflammation and contribute to age-associated comorbidities.
Alcohol use may accelerate biological aging.

Objective and Significance

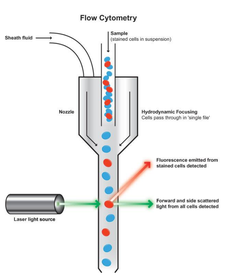
This preliminary study aims to develop and apply a flow cytometry–based assay to identify senescent immune cell and associated IFN-g expression. Our long-term goal is to identify whether alcohol use increases markers of cellular senescence in older adults.

Methods

- Peripheral blood mononuclear cells (PBMCs) from blood bank donors were used for optimization of assay
- Pilot study using PBMCs from 6 individuals aged 60 and older were used to test the validity of the assay.
- Low Alcohol = AUDIT-C < 3
- High Alcohol = AUDIT-C >3
- Staining for Tag-it, CD8, CD45, CD38,
- CD14, CD20, VIAB, CD4, CD28, CD3,
- Ki-67, IFN-g, and Beta-galactosidase
- Controls included unstained sample,
- single-stained samples, and Fluorescence
- Minus One controls (FMOs).



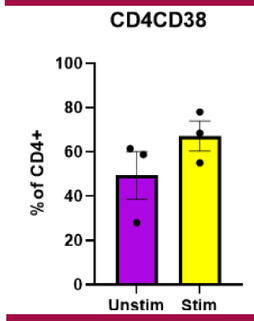
Cells are thawed and rested overnight → Stimulation cocktail (10 ng/mL PMA and 1 µg/mL) added and cells are incubated overnight → GolgiBlock added and cells are incubated for 4 hrs. After incubation, cells are stained for flow cytometry



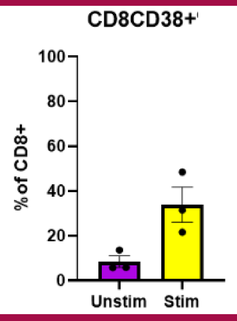
Flow cytometry analysis was performed using Cytek Northern Light.

Results

Assay Validation

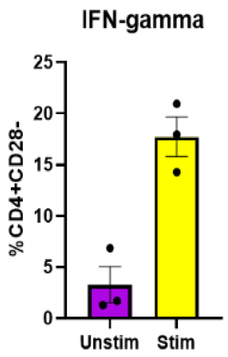


Stimulation increases CD38 expression in CD4 cells.

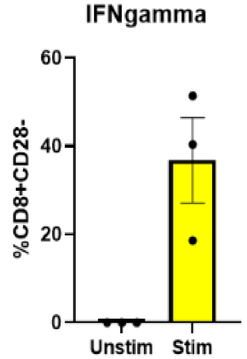


Stimulation increases CD38 expression in CD8 cells.

Assay Validation: IFN-gamma Expression



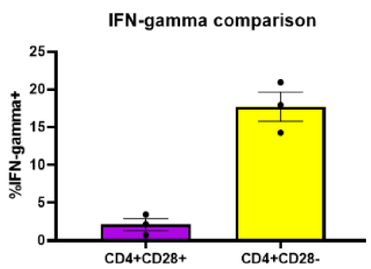
Stimulation increases IFN-gamma expression in CD4 cells. The largest increase was in CD4+CD28- cells.



Stimulation increases IFN-gamma expression in CD8 cells. The largest increase was in CD8+CD28- cells.

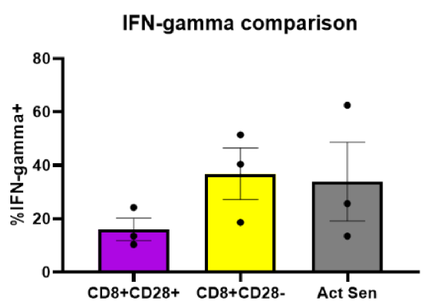
CD4 Sub-pop Comparison

* Showing stim groups only



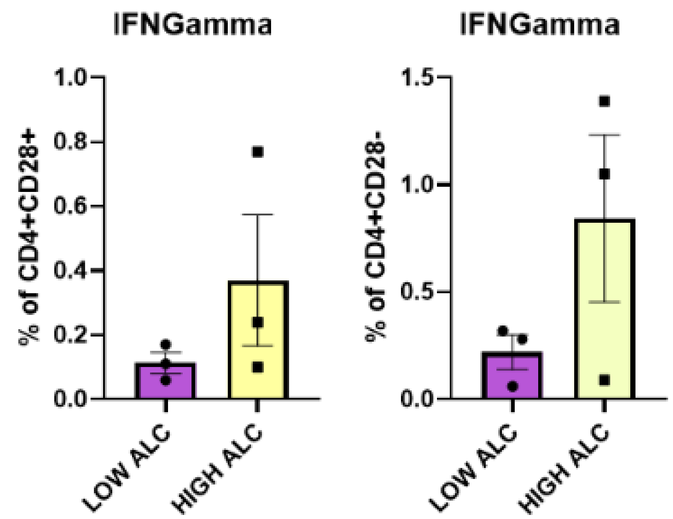
Senescent CD4+CD28- cells show a larger increase in IFN-gamma expression.

CD8 Sub-pop Comparison



Senescent CD8+CD28- cells show a larger increase in IFN-gamma expression.

IFN-gamma Expression in CD4 T Cells from Older Adults



Preliminary data indicates higher frequency of IFN-gamma in CD4 cells without stimulation in PBMCs from older adults with high alcohol use.

Discussion

- Optimized an in-vitro cytokine stimulation assay using the blood bank donor cells
- Stimulation of PBMCs increased IFN-g in both CD4 and CD8 T cells, especially in senescent and activated senescent cells
- Preliminary analyses of PBMCs from older adults with high alcohol use have increased IFN-gamma in CD4 cells.
- Ongoing studies will confirm these results in a larger sample size and associate the immune cell phenotype with onset of age-associated physical decline and increased risk of disease

Conclusion

- Optimized a flow-cytometry based in-vitro stimulation assay to assess cytokine profiles in immune cells including aging cell phenotypes
- Alcohol use in older adults potentially increases IFN-gamma expression in CD4 T cells, suggesting a pro-inflammatory phenotype

Acknowledgements

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epiMAP Study