Optimizing Cytokine Cocktails in Pancreatic Islet Organoids to Investigate Maturation-Related Vulnerability to Demise from Pancreatic Islet-Directed Autoimmunity

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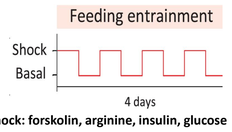
Introduction

- Type 1 Diabetes (T1D) is an autoimmune disease affecting over 8 million people worldwide. During disease progression, autoimmune attack leads to the demise of the insulin-producing beta cells of the pancreatic islet.
- It was recently uncovered that T1D is associated with early onset innate autoimmunity that precedes the adaptive autoimmune response.
- The link between islet maturation and vulnerability to innate autoimmune attack is unclear; does an immature phenotype protect beta cells from autoimmune attack, or does autoimmune attack induce a de-differentiated phenotype?
- Direction of causality can be determined by treating a pancreatic islet organoid model system with a cytokine cocktail that models autoimmune attack at varying stages of organoid maturity.

Objective: Optimize a cytokine cocktail for use on a pancreatic islet organoid model system.

Background

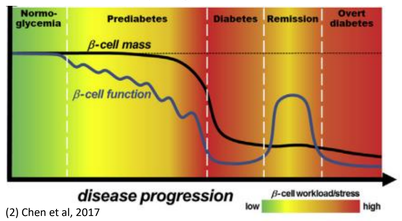
I. Pancreatic islet organoid model system: differentiation & entrainment



(1) Alvarez-Dominguez et al, 2020

hPSCs are differentiated into immature islets, which can be matured by entrainment to a circadian feed-fast cycle.

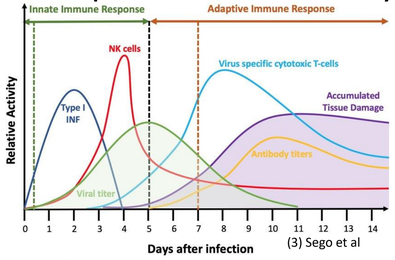
II. T1D disease progression



(2) Chen et al, 2017

Preclinical Asymptomatic phase begins with a precipitating event for autoimmune onset. Beta cells lose function but do not die until symptomatic phase.

III. Adaptive vs. Innate Autoimmunity



(3) Sego et al

Both adaptive and innate responses are involved in islet-directed autoimmunity

IV. Early innate autoimmune response is transient & precedes adaptive immunity

Type 1 Interferon (IFN) signaling has been detected prior to the appearance of autoantibodies (associated with adaptive immune response) but decreases after autoantibody seroconversion (4). Innate immune signaling is characterized by cytokine/IFN pathways that trigger apoptosis or recruit phagocytic cells.

V. Innate immune attack may be attenuated with beta-cell maturity

Surviving populations of pancreatic beta cells in T1D patients display an immature phenotype (5)

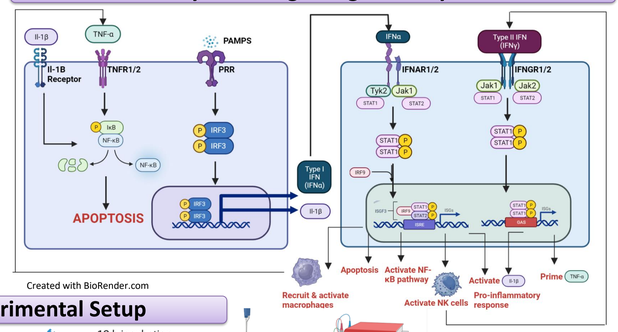
VI. Maturation-related demise might occur through increased beta cell secretory burden and thus enhanced ER stress (6)

Methods

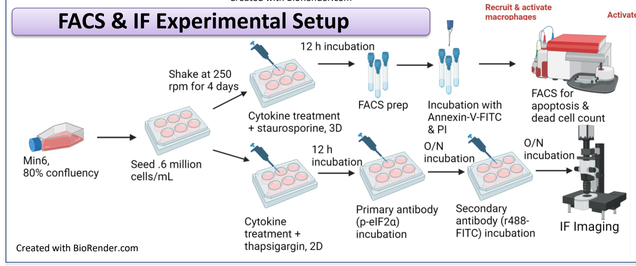
Cytokine Cocktail Selection

(1) IFNα (2000 U/mL) + Il-1β (50 U/mL)
(2) IFNγ (1000 U/mL) + Il-1β (50 U/mL)
(3) TNFα (1000 U/mL) + IFNγ (1000 U/mL)

Cytokine Signaling Pathways



FACS & IF Experimental Setup



Min6 (a mouse insuloma pancreatic beta cell line) cells were used for initial optimization experiments to minimize costs

Results

Cytokine cocktail of IFNα + Il-1β or IFNγ + Il-1β does not induce cell death

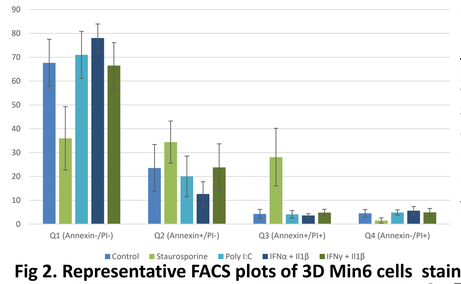
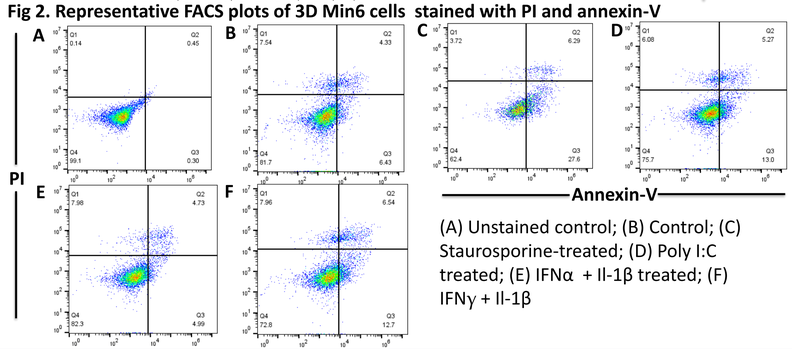


Fig 1. Quantification of annexin-V and propidium iodine (PI) staining with fluorescent-activated cell sorting (FACS) in 3D Min6 cells. Cells were treated with staurosporine (positive control for cell death), poly I:C (positive control for interferon signaling), IFNα + Il-1β, or IFNγ + Il-1β. N = 3 biological replicates. Quadrants were assigned as follows:

Q4	Q3
Q1	Q2

Fig 2. Representative FACS plots of 3D Min6 cells stained with PI and annexin-V

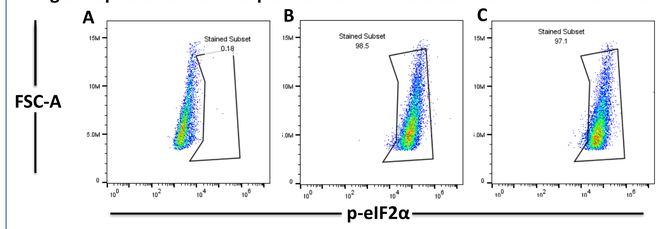


(A) Unstained control; (B) Control; (C) Staurosporine-treated; (D) Poly I:C treated; (E) IFNα + Il-1β treated; (F) IFNγ + Il-1β

Results

FACS unable to quantify ER stress markers in 3D Min6 Cells

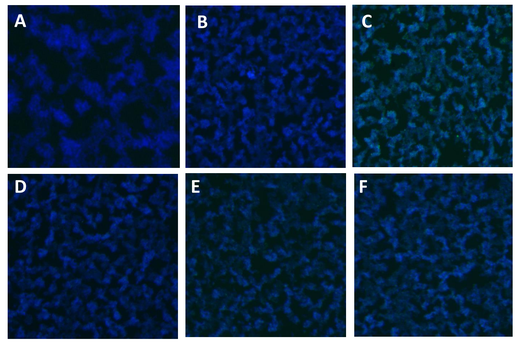
Fig 3. Representative FACS plots of 3D Min6 cells stained with ER stress marker p-eIF2α



(A) Unstained control
(B) Stained control
(C) Thapsigargin-treated

Cytokine cocktail of IFNα + Il-1β or IFNγ + Il-1β induces ER stress

Fig 4. Immunofluorescence images of 2D Min6 cells after cytokine treatment. Stained for DAPI in blue and ER stress marker p-eIF2α in green. (A) Thapsigargin-treated, secondary-only incubation; (B) Control; (C) Thapsigargin-treated; (D) Poly I:C treated; (E) IFNα + Il-1β treated; (F) IFNγ + Il-1β treated.



Conclusions & Future Directions

- 3D Min6 cells are unresponsive to the IFNα + Il-1β and IFNγ + Il-1β cytokine cocktails in terms of cell death, but do respond with increased ER stress. An optimal cytokine cocktail cannot yet be determined. Future optimization experiments will include the TNFα + IFNγ cocktail and will be performed in islet organoids instead of Min6 cells.
- Poly I:C incubation fails as a positive control for interferon signaling. Future experiments will use Poly I:C transfection to account for intracellular receptors.
- Once an optimized cocktail is determined, future experiments will involve treatment of entrained islet organoids to model vulnerability to immune attack at varying stages of maturity

Acknowledgements

Thank you to the Alvarez lab for hosting me this summer, to Dr. Juan Alvarez for providing guidance throughout the project, and to Zihan Gao and Mai Liu for help with relevant techniques. Thanks also to the Conn & Simon labs for generously gifting antibodies used in this project.

References