Determining the Tolerogenic Capacity of Trophoblast-Derived Extracellular Vesicles (EVs) on Macrophage Cytokine Secretion

Jenna Materna, B.S.E Biomedical Engineering (Biological Devices)
Mentor**:** Dr. Jessica Weaver, PhD, Assistant Professor
School of Biological and Health Systems Engineering (SBHSE)

Background

Type 1 Diabetes Mellitus (T1D) is an autoimmune disorder that impairs pancreatic islet function. Islet cell transplantation is a promising treatment, but direct and indirect antigen recognition lead to rejection by the immune system. This immune intolerance necessitates the use of toxic immunosuppressive therapies which harbor a host of health complications. Literature suggests that trophoblast extracellular vesicles (EVs) contain immunomodulatory factors that play a role in fetal immune tolerance, which can be used to develop tolerogenic therapies. Developing strategies to utilize immunomodulatory factors can lead to the development of alternatives to toxic immunosuppressant therapies currently used in conjunction with cell transplant recipients.

Objective

This study aims to determine the immunomodulatory potential of trophoblast-derived extracellular vesicles (EVs) on macrophages in vitro by tracking cytokine release by M0 macrophages over time using ELISA. ELISAs will be ran to determine the presence of 3 different prevalent immune markers including IL-6, LI-10, and TNF-alpha. This data will be used to support the proposition that extracellular vesicles may serve to induce tolergenicity in cell therapeutics.

Cytokines of Interest

IL6
a pro-inflammatory cytokine

IL10
an anti-inflammatory

TNFα
a pro-inflammatory cytokine

Acknowledgments

I would like to thank Dr. Weaver and the rest of the Weaver Lab for the opportunity to contribute to their work and this project. Special thanks to my graduate student mentor Shivani Hiremath.

Methods

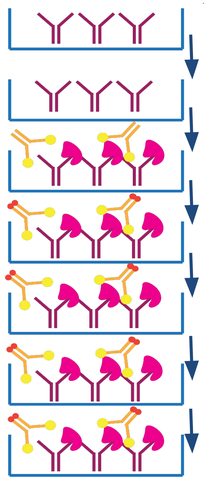
Day 1/2
1. Passage JAR cells and allow to grow

Day 3
1. Warm THP-1 CCM
2. Replace JAR CCM with THP-1 CCM for 8 hours
3. Collect JAR CCM, centrifuge at 2000xg for 15 min, and store at -80
4. After 8 hours, aspirate THP-1 media and replace with warmed JAR media

Day 4
1. Collect CCM from JAR flask, centrifuge at 2000xg for 15 min
2. Collect supernatant into 100kDa MWCO centrifuge tube and spin at 3000xg for 15 min and collect retenate in new tube
3. Passage JAR cells
4. Initiate EV isolation by adding total exosome isolation reagent at half the volume of retenate and incubate at 4C overnight

Day 5
1. Centrifuge at 10000xg for 1 hour at 4C
2. Discard supernatant and resuspend pellet
3. Quantify EVs with BCA assay
4. Place EVs in 4C overnight

Day 6
1. Treat M0s with JAR EVs at 2 and 6 hours at 30 ug/mL
2. Collect supernatants from each time point and store at -80C



ELISA Assay
1. Coat 96-well half-volume plate with 50 uL diluted Capture Antibody and incubate overnight between in 4C.
2. Wash 4x, add 100 uL 1X Assay Diluent A, incubate for 1 hour at RT while shaking.
3. Wash 4x, add 100 uL diluted standards and samples, incubate for 2 hrs at RT while shaking.
4. Wash 4x, add 100 uL diluted Detection Antibody, incubate for 1 hr at RT while shaking
5. Wash 4x, add 50 uL Avidin-HRP, incubate 30 min at RT while shaking.
6. Wash 5x, add 50 uL TMB Substrate Solution (A+B), include 15 min at RT while covered.
7. Add 50 uL stop solution.
8. Read absorbance at 450nm and 570nm.

ELISA Results

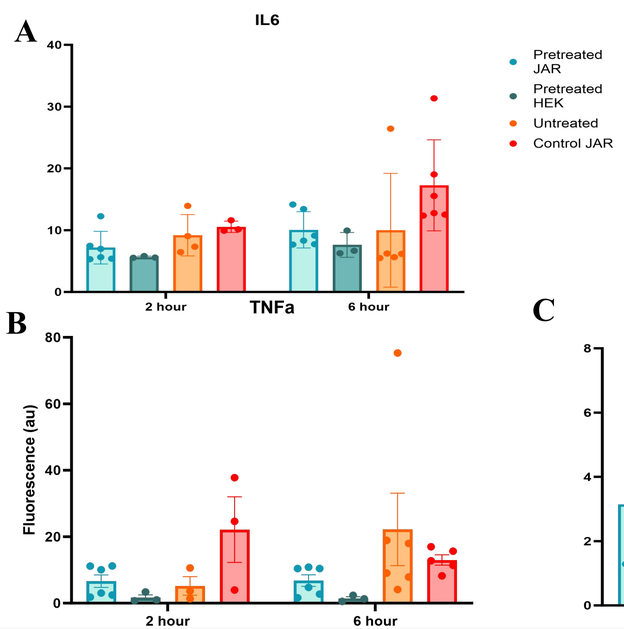


Fig 1. (A) ELISA analysis of TNFa cytokine production by pretreated JAR, pretreated HEK, untreated, and control JAR cells. **(B)** ELISA analysis of IL-6 cytokine production by pretreated JAR, pretreated HEK, untreated, and control JAR cells. **(C)** ELISA analysis of IL-10 cytokine production by pretreated JAR, pretreated HEK, untreated, and control JAR cells.

Results

The ELISA analysis of cell culture media of cells pretreated with extracellular vesicles (EVs) demonstrates a suppression of pro-inflammatory cytokines and a possible upregulation of inflammatory cytokines. TNFa and IL6 were both reduced between the 2 and 6 hour time points in the EV treated groups, evidencing a potentially tolerogenic response of polarized cells to EV exposure.There was some elevation in IL10 levels between the 2 and 6 hour time marks in the EV treated groups as well, indicating the possible upregulation of anti-inflammatory cytokines in response to EV exposure. The combination of the upregulation of anti-inflammatory cytokines and the decrease in pro-inflammatory cytokines in response to EV exposure supports the efficacy of EVs in the development of potentially tolerogenic therapeutics.

References

[1] Cleveland Clinic, “Type 1 Diabetes: Causes, symptoms, complications & treatment,” Cleveland Clinic, Mar. 09, 2022. https://my.clevelandclinic.org/health/diseases/21500-type-1-diabetes
[2] Mayo Clinic, “Type 1 Diabetes - Symptoms and Causes,” Mayo Clinic, Sep. 15, 2023. https://www.mayoclinic.org/diseases-conditions/type-1-diabetes/symptoms-causes/syc-20353011
[3] National Institute of Diabetes and Digestive and Kidney Diseases, “Pancreatic Islet Transplantation | NIDDK,” National Institute of Diabetes and Digestive and Kidney Diseases, Feb. 28, 2019. https://www.niddk.nih.gov/health-information/diabetes/overview/insulin-medicines-treatments/pancreatic-islet-transplantation
[4] UCSF, “Transplant Surgery - Islet Transplant for Type 1 Diabetes,” Ucsf.edu, 2019. https://transplantsurgery.ucsf.edu/conditions--procedures/islet-transplant-for-type-1-diabetes.aspx
[5] “Immunosuppressants,” Cleveland Clinic. https://my.clevelandclinic.org/health/treatments/10418-immunosuppressants#risks-benefits
[6] S. C. Hiremath and J. D. Weaver, “Engineering of Trophoblast Extracellular Vesicle-Delivering Hydrogels for Localized Tolerance Induction in Cell Transplantation,” Cellular and Molecular Bioengineering, vol. 16, no. 4, pp. 341–354, Aug. 2023, doi: https://doi.org/10.1007/s12195-023-00778-8.

FURI

Ira A. Fulton Schools of Engineering
Arizona State University