In-Vitro Recapitulation of NOD Murine Gestational Tolerogenic Immune Niche Using Splenocytes & JAR Trophoblast Cell Line

Omar Al Bukhari[1], Ariana Belton[1], Jessica Weaver, Ph. D[1]
[1]Tempe, AZ, School of Biological and Health System Engineering, Arizona State University

Ira A. Fulton Schools of Engineering
Arizona State University
SBHSE | School of Biological and Health Systems Engineering

Introduction

- Pregnancy (gestation) induces a tolerogenic/suppressive immune niche preventing rejection of genetically semi-allogeneic, implanted fetus [1,2]
- Induced immune mechanisms of pregnancy could allow for other useful outcomes➔ **Improved Biomedical Implant Resolution [3]**, **Delayed Type 1 Diabetes (T1D) Onset [4]**, etc.
- T-Cell (TC), B-Cell (BC), Natural Killer (NK), and Dendritic Cell (DC) involved in both T1D progression (Activated Phenotypes) [9], as well as generating a suppressive/tolerogenic immune niche during pregnancy (Suppressive Phenotype) [10]
- *The study examines the potential shift **in-vitro** of the immune niche towards tolerogenicity by co-culturing challenged (Post-T1D Onset) NOD Mouse Splenocytes with a Placental JAR cell-line*
- Results will provide insight into the potential mechanism and cell specific shift in T-Cell and NK phenotypes when cultured in-vitro with a placental cell line ➔ Provides a basis of understanding with regards to induced immune tolerogenic environment during gestational stages

Methods

- Preserved, Isolated Splenocytes from post-onset Female NOD's revived and co-cultured with JAR cell line at a 10:1 (Splenocyte:JAR) cell ratio ➔ Female splenocyte and JAR group (Experimental) and Female Splenocyte & Sham Spiral Group (Control)
- 1.5% Alginate $CaCO_3$ prepared and used to encapsulate JAR Trophoblasts during co-culture:
  - 3% Alginate (dissolved overnight), 100mM Glucono Delta-Lactone (GDL), and 30mM $CaCO_3$ mixed separately with (-)(-)Dulbecco's Phosphated Saline Buffer (DPBS)
  - 0.45M JAR cells prepared and then all Alginate reagents mixed to produce encapsulated spiral containing JAR cells
- Flow Cytometry Staining of Splenocytes for TC and NK markers to determine potential differences in immune niche ➔ Gating strategy pre-planned, and compensation, statistics, flow data analysis all performed in FlowJo 10/11, GraphPad Prism

Immune Cell Type	Cell Marker(s) Used
T-Cell	CD3e, CD4, CD8a, FOXP3, GATA-3, T-bet, CD44, CD62L, CD45
NK-Cell	CD56, CD107a, CD38, CD16, CD27, 159a KLRC1, CD45, CD3e

Table 1. General Cell Specific Markers Used for Each Immune Cell Type. This table describes some cell-specific immune markers that were used during stages of this study to determine immune cell phenotypes and subpopulations; i.e. during flow cytometry.

Results

NK Cell Panel Flow Cytometry:

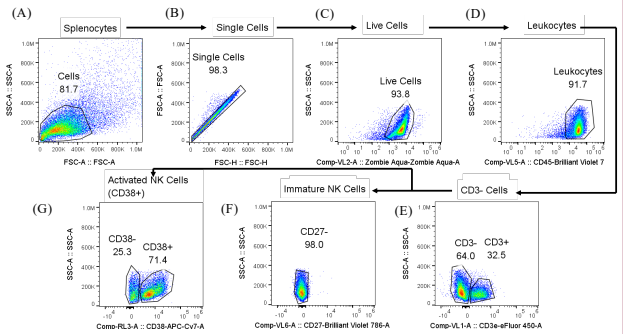
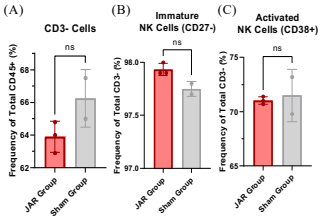


Figure 1. Example Gating Strategy Used in NK Panel Data. The graphs in this figure were produced using a replicate from the "Female JAR" group in which the scatter plots within the figure represent the general gating strategy for (A) Splenocytes (B) Single Cells (C) Live Cells (Rough Zombie Aqua Gate) (D) Leukocytes (CD45+) (E) CD3- Cells (F) Immature NK Cells (CD27-) & (G) Activated NK Cells (CD38+)



No significant differences between Sham vs. JAR Group NK Cell subpopulations (Figure 2)

Figure 2. Frequency of Different Murine NK Markers vs. Total Parent Populations. Bars indicate the mean frequency (%) of (A) CD3- compared to the total parent population of CD45+ cells and (B) Immature NK Cells (CD27-) & (C) Activated NK Cells (CD38+) compared to the parent population of CD3- cells. These were calculated using acquired flow data of "Female Sham" (n=2) and "Female JAR" (n=3) groups. Error bars represent standard deviation, and ns represents no significance between the groups.

T-Cell Panel Flow Cytometry:

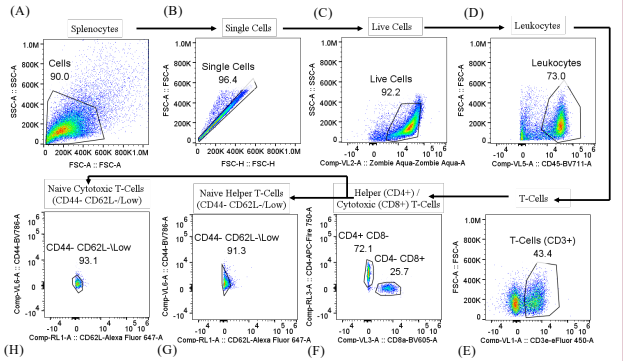


Figure 3. Example Gating Strategy Used in T-Cell Panel Data. The graphs in this figure were produced using a replicate from the "Female JAR" group in which the scatter plots within the figure represent the general gating strategy for (A) Splenocytes (B) Single Cells (C) Live Cells (Rough Zombie Aqua Gate) (D) Leukocytes (CD45+) (E) T-Cells (CD3+) (F) Helper (CD4+)/Cytotoxic (CD8+) T-Cells, (G) Naïve Cytotoxic T-Cells (CD8+ CD44- CD62-/Low), & (H) Naïve Helper T-Cells (CD4+ CD44- CD62-/Low)

Results

Sig. Differences in Helper (CD4+), Cytotoxic (CD8+), and Naïve Helper T-Cells (CD4+ CD44-CD62L-)

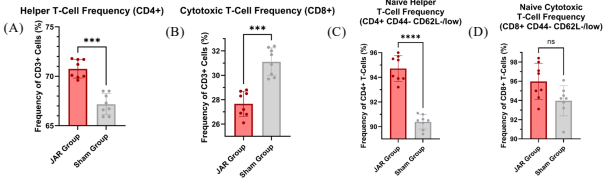


Figure 6. Frequency of Different Murine T-Cell Markers vs. Total Parent Populations. Bars indicate the mean frequency (%) of (A) Helper T-Cells (CD4+) & (B) Cytotoxic T-Cells (CD8+) vs. Total T-Cell Population (CD3+) as well as (C) Naïve Helper T-Cells (CD4+ CD44- CD62L-/Low) vs. Total Helper T-Cell Population (CD4+) & (D) Naïve Cytotoxic T-Cells (CD8+ CD44= CD62L-/Low) vs. Total Cytotoxic T-Cell Population (CD8+). These were calculated using acquired flow data of Sham (n=8) and JAR (n=8) groups. Error bars represent standard deviation, the increasing total number of * represents increasing statistical significance and ns represents no significance between the groups. A two-tailed paired parametric t-test ($p<0.05$) was performed on all data sets within this figure.

Summary, Conclusions and Future Directions

Potential shift towards a tolerogenic immune niche in the T-Cell data specifically ➔ **Statistically significant increase in Helper & Naïve Helper T-Cells, and Decrease in Cytotoxic T-Cells in JAR Group**; **Consistent with other pregnancy studies [7,8]**

Study generally showed that an in-vitro gestational model could be possible with improvements ➔ Larger sample size, include target MHC-I Cells to mimic pregnancy [9], run at different timepoints, etc.

In the future, this study could include more groups: *male NOD groups, mixed genotype NOD mouse groups, splenocytes from JAR treated mice, etc.* Furthermore, the in-vitro study should be expanded to include a BC and DC panel to better understand the larger immune interaction involved. Finally, in the far future, this should be repeated in-vivo to confirm physiological accuracy in NOD Mice.

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Acknowledgements

I'd like to thoroughly acknowledge Ariana Belton for allowing me to use her cell samples for my project, as well as guiding me through the most nuanced portions of this project. Furthermore, I'd also like to extensively thank Dr. Weaver for giving me the opportunity to work in her lab and giving me the freedom and independence (with limits of course) to decide the best course of action for this study.