Cytokines drive T cell proliferation while maintaining low levels of activation markers

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Introduction

Type 1 Diabetes is an autoimmune disease characterized by T-cell mediated destruction of pancreatic islet beta cells, leading to insulin deficiency.[1] One possible treatment is novel antigen-specific immunotherapy—using specially-engineered tolerogenic antigen-presenting cells (APCs) to reprogram autoreactive T cells and restore their tolerance. Engagement with tolerogenic APCs activates the T cell without presenting the necessary costimulatory molecules, leading to T cell anergy (inactivation).[2,3] To characterize this process in vitro, human primary T cells must be transduced with autoreactive T cell receptors, which requires cell proliferation.

Anti-CD3/CD28 are known to drive extensive proliferation via direct T-cell receptor (TCR) stimulation, but also upregulate activation markers, making it difficult to monitor subsequent activation and reprogramming by APCs.[4] Cytokines (IL-7, IL-15, IL-2, IGF-1), on the other hand, have been shown to drive comparatively lower levels of proliferation without significantly upregulating activation markers.[5]

Here, we performed proliferation assays and measured activation marker (CD25, CD69) fluorescence over a time period of about two weeks. By comparing these data, we aim to identify the optimal conditions for transduction while maintaining sufficiently low levels of activation markers.

Materials and Methods

- Peripheral blood mononuclear cells (PBMCs) were isolated from human blood, and T cells were subsequently purified from PBMCs by magnetic separation
- Cells were stained with CFSE (proliferation dye)
- **Conditions**
  - "Beads"—Anti-CD3/CD28 beads (removed after 3 days)
  - "ILs"—IL-7, IL-15, IL-2
  - "Mix"—IL-7, IL-15, IL-2, IGF-1
- **Timepoints:** Day 0, 3, 5, 7, 10, 13

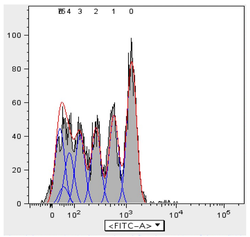

Figure 1: CFSE Proliferation assay

Analysis Panel
PI (viability dye)
CD4 (PE)
CD8 (PB)
CD25 (APC)
CD69 (PECY7)
CD45RA (BV510)
CD45RO (APC/CY7)
CD28 (BV605)
CD154 (BV711)

Results

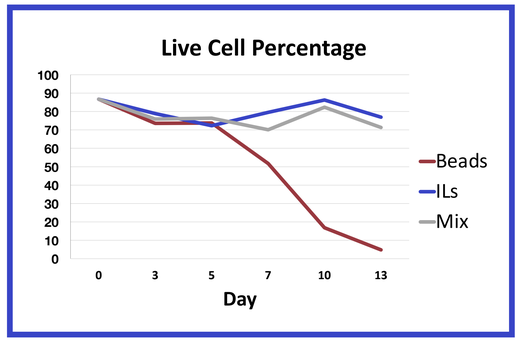

Figure 2: Cell viability over two weeks by treatment type
Cells treated with beads experienced a rapid decline in viability after day 5, while cells treated with cytokines maintained a steady level throughout (~80%)

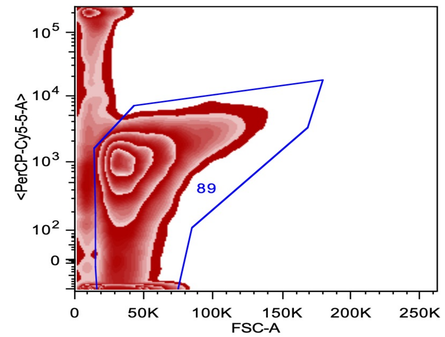

Figure 3: Flow cytometry Zebra Plot (Mix, Day 5)
Cells gated in blue (89%) are live cells. Dead cells are stained with propidium iodide

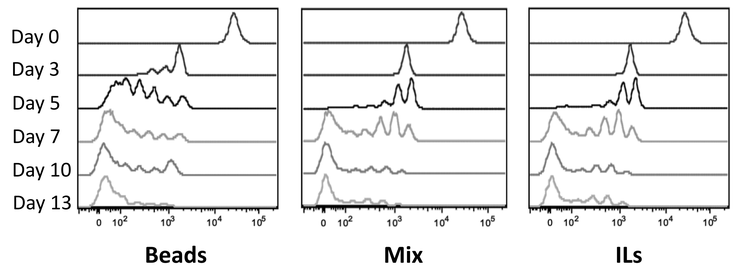

Figure 4: CFSE Proliferation assay for CD8 T cells over two weeks by treatment type
Each peak represents a new division; the more peaks, the more cells that have proliferated.

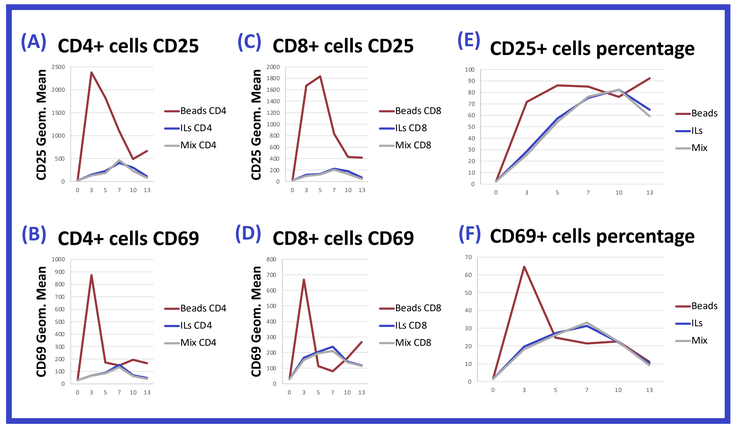

Figure 5:
(A)-(D):
CD25 & CD69 fluorescence levels for CD4 and CD8 T cells by treatment type over time
Activation marker levels rose and fell drastically with beads treatment, while they remained low for cytokines treatment.

(E)-(F):
Percentage of CD25+ and CD69+ cells by treatment type over time

Conclusions

- Viability remained high for cells treated with cytokines, but started decreasing around Day 5-7 for cells treated with beads
- Cytokine treatments induced proliferation with a slight delay compared to beads treatment, but resulted in almost the same level of proliferation by Day 13 of culture
- While anti-CD3/CD28 significantly upregulated activation marker levels, CD25 and CD69 levels remained low throughout the experiment for cytokines treatment, which is helpful for testing subsequent activation and reprogramming by tolerogenic APCs.

Future Directions

- Validate autoreactive TCRs
- Transduce validated TCRs into human primary under the optimal conditions identified in this experiment to further test tolerization by APCs

References

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Acknowledgments

I would like to thank Dr. Remi J. Creusot, Dr. Nato Teteloshvili, Dr. Rebuma Firdessa Fite, and Dr. Jorge Postigo-Fernandez for their guidance and mentorship. I would also like to thank the URF and Laidlaw Foundation for their generous support of this research.

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