Process Optimization of Multiple Lymphocyte Reaction in Immunotherapy

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Mixed Lymphocyte Reaction (MLR) in Immunotherapy

MLR measures the immune response of T cells when mixed with antigen presenting cells from two different blood donors. This is used to determine whether therapeutic antibody candidates can enhance immune response.

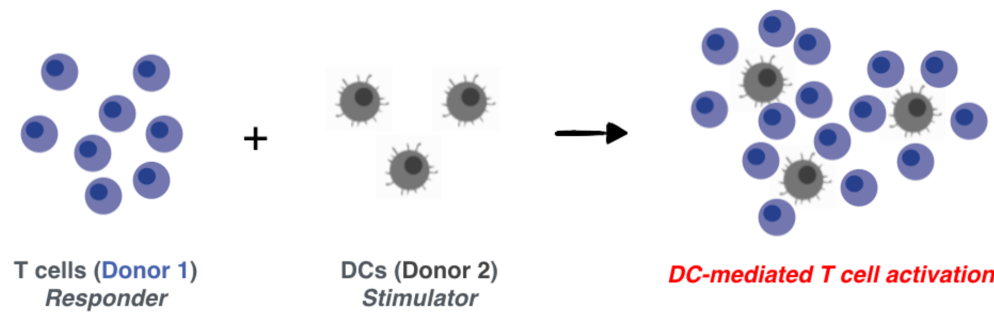

Fig. 1 Mixed Lymphocyte Reaction

Objective: Optimize the efficiency and range of data acquired from MLR, using high throughput flow cytometry.

Flow Cytometry

Flow cytometry is a technique used to characterize populations of fluorescently labeled cells based on light scatter measurements.

Current Drawbacks

- Only one time point data obtained
- Takes 3+ weeks to run the experiment and analyze data
- Only measures cytokines
- Limited ability to test different cell types activation status

Fig. 2 Sartorius iQue3 High Throughput Flow Cytometer

Sartorius iQue3 High Throughput Flow Cytometry

- Rapid analysis of cells surface antigens, cytokine secretion, activation and survival
- Requires smaller sample volumes
- Customizable and adaptable data analysis

Methods

Peripheral blood mononuclear cells (PBMC's) were isolated from two different human blood donors. PBMC's from Donor 1 were stimulated with GMCSF and IL-4 to induce DC differentiation. On day seven, two populations of CD4+ cells (total and resting) are isolated from Donor 2 blood. DC's from Donor 1 were then combined with T cells from Donor 2 along with antibodies capable of triggering the anti-cancer immune response. Markers of T cell activation such as cytokine production, viability, and proliferation are measured over eight days using the iQue3 flow cytometer.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Donor #1	Isolate Monocyte	Culture Monocyte: Expand to Dendritic Cells					Detach DCs	MLR: Culture DC with CD4+ Measure cytokine secretion, surface markers, cell survival, and T cell proliferation				
Donor #2							Isolate CD4+					

Fig. 3 MLR Timeline

	1	2	3	4	5	6	7	8	9	10	11	12
A	Total CD4+						Resting CD4+					
B												
C		Media Only		Antibody 1				Media Only		Antibody 1		
D		T Cells Only		Antibody 2				T Cells Only		Antibody 2		
E		DCs Only		Antibody 3				DCs Only		Antibody 3		
F		T + DC		Antibody 4				T + DC		Antibody 4		
G				Activated T						Activated T		
H												

Fig. 4 MLR Assay Plate Map

"Activated T" represents a positive control condition in which T cells are preactivated with activator beads that mimic T cell activation from antigen presenting cells by utilizing CD3 and CD28 signals.

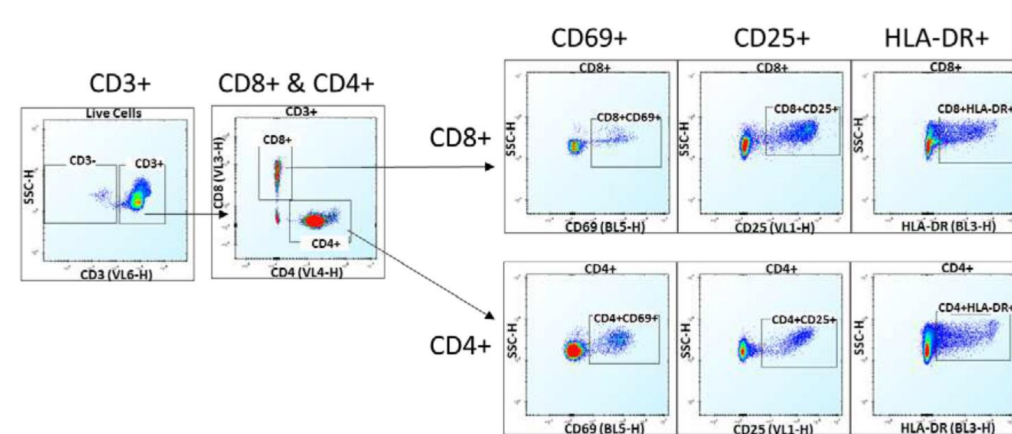

Fig. 5 Cell Gating on iQue Flow Cytometer
Cell populations are distinguished based on light scatter of different colored lasers within the flow cytometer

Results

Cell Survival

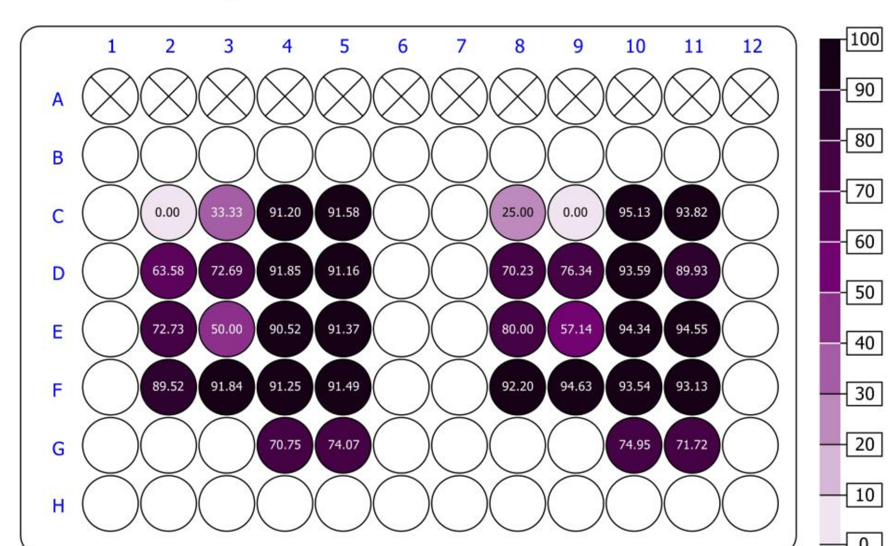

Fig. 6 Cell Survival Heat Map

Results

CD4+ T Cell Activation & Cytokine Secretion

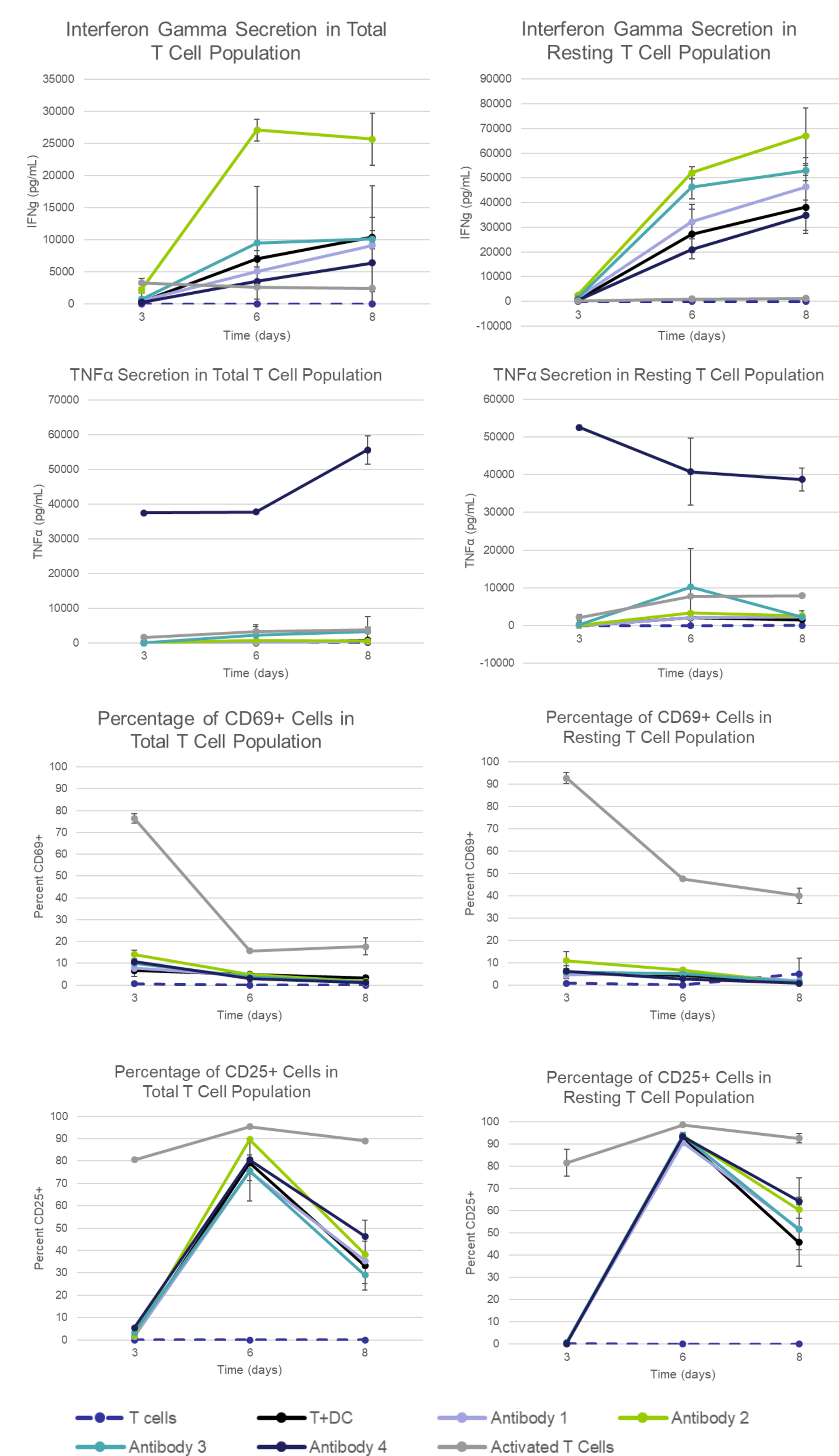

Fig. 7 Time Dependent T Cell Activation and Cytokine Secretion
n=2 for all graphs; Error bars represent standard deviation
Data points outside the linear range of standard curves were excluded from graphs.

Cell Proliferation

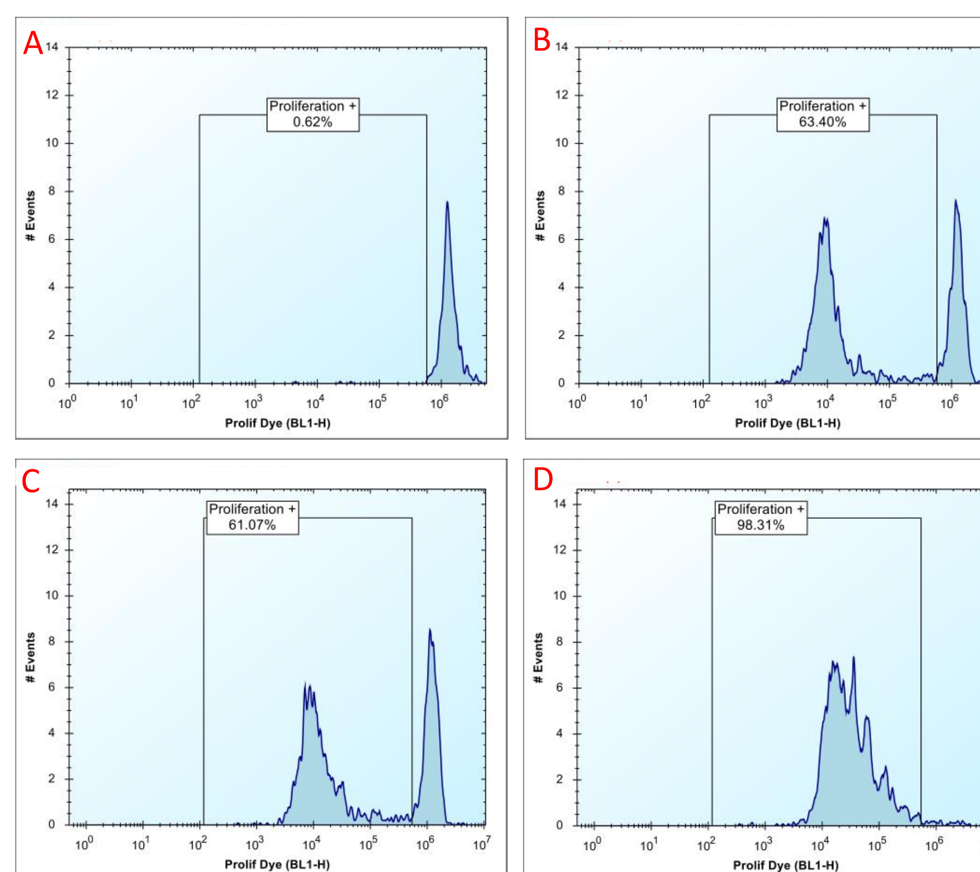

Fig. 8 Cell Proliferation+ Populations on Day 6 Culture
(A) T cells only (negative control) (B) T cells + DC (C) T cells + DC + Antibody 1 (D) Pre-activated T cells (positive control)

All T cells were stained with proliferation dye prior to the MLR assay. As cells proliferate, concentration of the dye within each cell decreases. Thus, lower fluorescence levels indicate higher levels of proliferation.

Analysis

- Cell survival is maintained through test populations and slightly decreased in preactivated T cells due to over stimulation.
- INFg secretion in test samples increases as expected over 8 days.
- TNFa secretion remains low in all test populations except the positive control.
- Maximum CD69 expression is observed earlier in the co-culture, as expected of an early activation marker
- CD25 reaches maximum expression on day 6.
- Resting CD4+ T cell populations showed a more robust activation response overall than total CD4+ T cell populations.

Conclusions

- High throughput flow cytometry suggests a more effective method of conducting MLR readout.
  - More time efficient
  - Wider range of data
  - Simultaneous measurements of different parameters of T cell activation
  - Small sample volume required
- These improvements are extremely useful in the field of immunotherapeutic discovery as drug efficacy can be tested more efficiently and new treatments can reach patients in clinical trials faster.

Next Steps

- Perform assay with triplicate conditions rather than duplicate to decrease error
- Include intermediate timepoints between days 3 and 7 for more specific transient data
- Test therapeutic antibody candidates

References

1) *Mixed Lymphocyte Reaction (MLR) Assays.* ProImmune. (2021).
2) McKinnon, K. M. (2018). Flow cytometry: An overview. *Current Protocols in Immunology*, *120*(1).

Figure 1. *Mixed lymphocyte reaction.* Explicyte.