T Cell Expansion Kinetics Dependence on Soluble Activator Additions

Ali Chamkalani[1,2] Yishuang Zhang[1,2], Bhushan Gopaluni[3], James Piret[1,2,3]

[1]Michael Smith Laboratories, [2]School of Biomedical Engineering, [3]Department of Chemical and Biological Engineering, The University of British Columbia, Vancouver, BC, Canada

Introduction

The approval of chimeric antigen receptor T-cell (CAR-T) therapies has been a major breakthrough for patients with relapsed or refractory blood cancers. To reach the desired clinical dose, T cell populations are expanded ex vivo by cell cultures with complex dynamics that present several challenges. The use of a soluble activator was investigated as a means to simplify the manufacturing process. The common view of T cell activation using beads is that the cells should be activated for only a few days, and that the subsequent presence of the activator is not required for expansion, and can even have negative effects. As the field seeks to develop sustainable manufacturing processes, it is important for their optimization to increase the understanding of the activator dependence of human T cell culture expansion.

Materials and Methods

T cells were isolated from the healthy donor's buffy coat using the RosetteSep™ Human T Cell Enrichment. Thawed T cells were activated with soluble CD3/CD28/CD2 T Cell activator on day 0, and expanded in ImmunoCult™-XF T Cell Expansion Medium.

On days 3, 5, 7, and 9,the cells were counted and passaged. Flow cytometry was performed to analyze T cell differentiation and exhaustion phenotype of expanded cells on the same day.

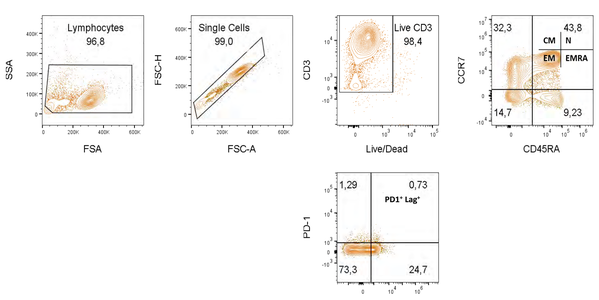

Figure 1. Representative flow cytometry data.

Results

Fold expansion and calculated activator dilutions

T cells were inoculated at 250,000 cells/mL and then activated using four different doses of CD3/CD28/CD2 soluble T cell activator, and then medium dilutions with fresh media on day 3 subsequently varied the activator medium levels to a greater extent.

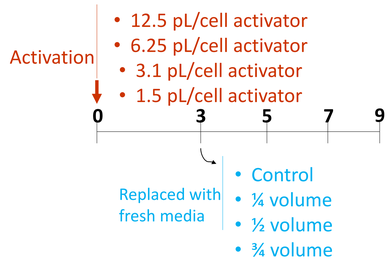

Figure 2. Schematic for T cell activation and dilution on days 0 and 3, subsequently.

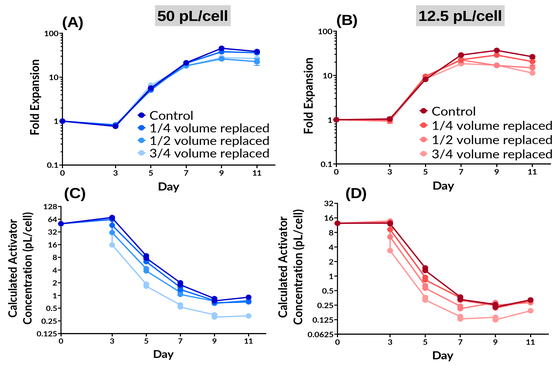

Figure 3. (A-B) Fold expansion and (C-D) calculated activator level kinetics for 2 initial doses of activator (50 and 12.5 pL/cell)

- On days 7 and 9, the control condition yielded higher fold expansion so that as the media gets diluted more, the cells enter the stationary phase sooner.
- For the higher initial dose (50 pL/cells), the time that cells grow in the exponential phase is longer resulting in higher fold expansion.

Maintaining sufficient activator levels results in high expansion and viability

Isolated T cells were seeded at 0.5 x 10^6 cells/mL, and soluble activators added at 25, 6.25, or 3.1 pL/cell on day 0.

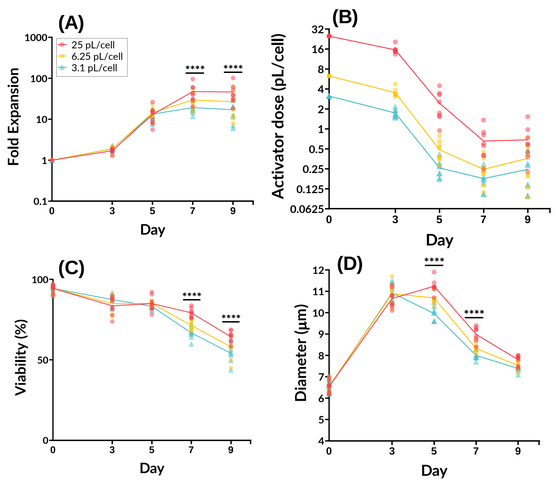

Figure 4. (A) Proliferation is lower for lower doses (B) calculated activator per cell which is correlated with growth (C-D) By day 5, the culture profile is the same due to sufficient activator. However, as the activator is limited per cell, the decline rate in viability and diameter increases

- By day 5, all cultures were similarly expanded. However, from then on, conditions with higher doses have a more prolonged exponential phase
- As viability decreases over time, higher activator doses showed higher viability after day 5.

Results

- Although cell size increases and then begins to decrease for all three conditions when cells are activated, the decrease in diameter is delayed for cultures containing higher doses.

The low dose yielded a comparative central/effective memory cell percentage with less expression of PD1 and Lag-3

- All three conditions have approximately the same % central memory (CM)
- and effective memory (EM) cells.
- For the 3.1 pL/cell condition, % CM is almost unchanged despite no net growth.
- Persistent stimulation leads to T cell exhaustion, and since the activator is still available in these cultures, this presence may result in exhaustion. Decreasing expression of PD1 and Lag-3 after day 3 indicating minimal exhaustion in all three conditions.
- At 3.1 pL/cell dose condition, T cells expressed lower PD1 and Lag-3 .

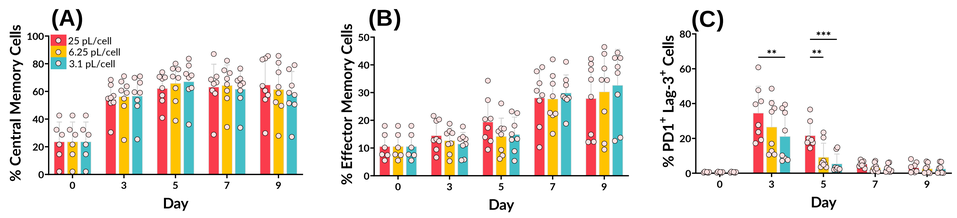

Figure 5. (A-B) Central memory and effector memory cell percentage of live CD3 cells, (B) the co-expression of inhibitory markers had a decreasing trend, indicating exhaustion .

Fold expansion was not dependent on the initial cell concentration but rather on the activator per cell

- Given the importance of activator per cell levels, we investigated the combinatory effects of the initial activator addition and the inoculation cell concentration.
- The expansion profile was the same for different seeding concentrations with the same activator dose.

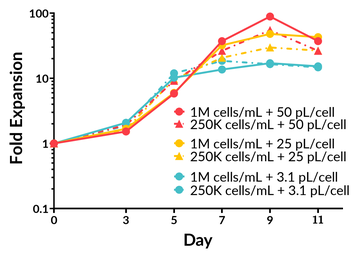

Figure 6. Regardless of the initial seed concentration, the growth curve depends on the activator per cell

Conclusion and Future Work

- T cell growth depended on the activator per cell additions.
- Maximal production was obtained when sufficient calculated activator levels remain present throughout the culture
- Methods are being developed to measure the soluble activator levels.
- An understanding of activator level influences is important for process optimization.
- This approach should also enable more cost-effective expansion of T cells, for ranges of initial cell concentration and restimulation timing.