

INTRODUCTION

T Regulatory Cells (Tregs) constitute a small subset of CD4⁺ cells and play a crucial role in treatment of autoimmunity. Thus, research or patient treatment with these cells requires scalable and efficient expansion methods. Current products on the market use plate bound antibody or antibody-coated magnetic beads for expansion. These methods pose challenges regarding scalability and the removal of magnetic beads before downstream applications, respectively. The Cloudz™ Human Treg Expansion Kit utilizes dissolvable hydrogel microspheres conjugated with anti-CD3 and anti-CD28 antibodies (Cloudz™ Treg CD3/CD28) to address these challenges by simplifying the scale-up and collection of cells for downstream applications. The microspheres provide a 3-dimensional surface area that increases cellular interaction during expansion. After expansion, the hydrogel microspheres can be dissolved rapidly and removed from culture. We present data demonstrating a working dose of Cloudz™ Treg CD3/CD28 that produces similar growth and FoxP3 (a Treg master transcription factor) expression levels as commercially available expanders. Furthermore, we show the scalability of Treg expansion by using Cloudz™ Treg CD3/CD28 in the G-Rex6M platform. The G-Rex platform is unique in that the plates have a membrane at the bottom that allows for gas exchange and pH buffering.

RESULTS

Identifying a Working Dose of Cloudz™ Treg CD3/CD28 to Achieve High Expansion and Purity of CD4⁺/CD25⁺/FoxP3⁺ Cells

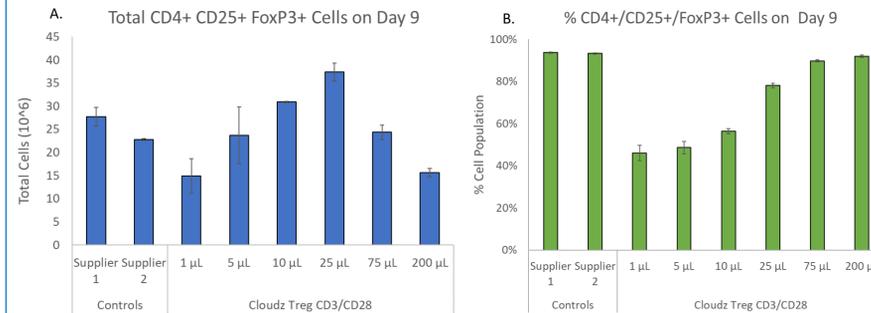


Figure 4. Dosing Assay using Cloudz™ Treg CD3/CD28. After 9 days of culture, a working dose of 75 μL Cloudz™ Treg CD3/CD28 produced a similar number (A) and percentage (B) of CD4⁺/CD25⁺/FoxP3⁺ cells when compared to both market suppliers. Cells were cultured in ExCellerate T Cell Media + 10% Fetal Bovine Serum (FBS) + IL-2. Data are presented as the mean ± S.D. (n=3).

Cloudz™ Treg CD3/CD28 Are Compatible With Serum-Free ExCellerate T Cell Media

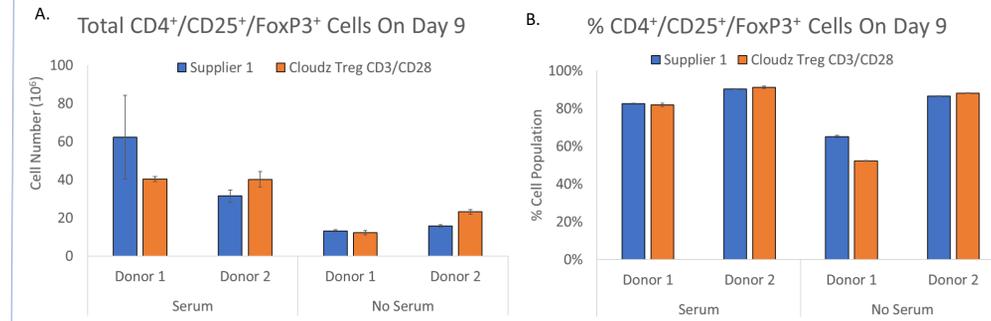


Figure 5. Growth and purity of CD4⁺ cells expanded with Cloudz™ Treg CD3/CD28 across two donors in serum-free ExCellerate media. Across 2 donors, CD4⁺ cells stimulated with Cloudz™ Treg CD3/CD28 performed similarly to Supplier 1 in both serum and no serum conditions. Serum-free media produced lower expansion across activators in both donors (A). The % CD4⁺/CD25⁺/FoxP3⁺ was similar with both activation reagents (B). Data are presented as the mean ± S.D. (n=3).

METHODS

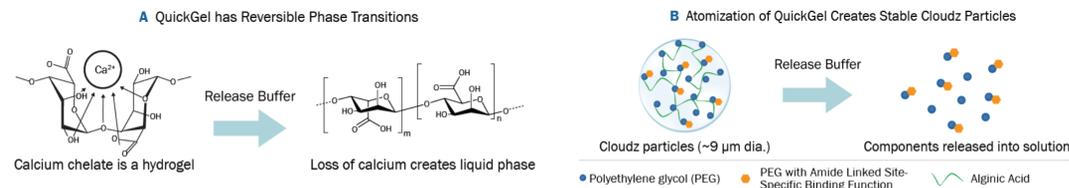


Figure 1. Generating Cloudz™ Particles using QuickGel. (A) QuickGel, the base material for Cloudz™ Treg microspheres, consists of alginate acid and polyethylene glycol prepared in the presence of calcium chloride. In the presence of chelating agents (Release Buffer), the hydrogel rapidly enters a liquid phase. (B) Using an atomization technique, stable hydrogel particles of approximately ~9 μm diameter are formed. These particles can be subsequently be functionalized by the addition of surface groups.

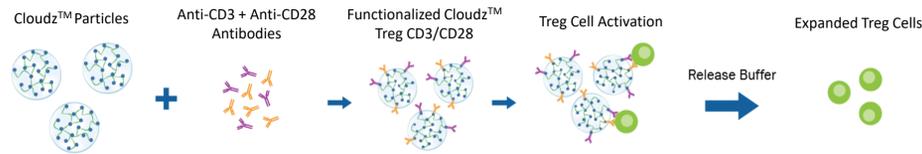


Figure 2. Functionalization of Particles to make Cloudz™ Treg CD3/CD28 For Expansion of Tregs of CD4⁺ Cells. Cloudz™ Treg CD3/CD28 was created by bioconjugating CD3 and CD28 antibodies onto the surface. CD4⁺ cells cultured with Cloudz™ Human Treg Expansion kit (R&D systems CLD-006) were started in 24 well plates with ExCellerate™ T Cell Media (R&D Systems CCM030) + human interleukin-2 (IL-2, R&D Systems 202-GMP). Cells were counted, characterized, and media was added every 2 days to maintain cell density. Positive control expanders were sourced from current market suppliers.



G-Rex6M Plate
Capacity: 100 mL
5e6 CD4⁺ Cells seeded per well

Refresh IL-2 Every 3 days

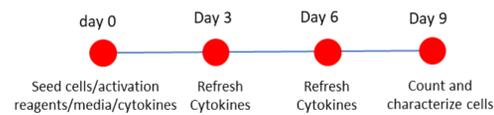


Figure 3. Culture Schematic for expansion of Tregs from CD4⁺ Cells in G-Rex6M plates using Cloudz™ Human Treg Expansion Kit. On Day 0, 5e6 CD4⁺ cells and 750 μL Cloudz™ Treg CD3/CD28 were cultured in G-Rex6M plates containing 100 mL of ExCellerate T Cell Media. 20 μL of IL-2 was added every third day (Day 0, 3, 6). Cells were counted and characterized on Day 0 and Day 9. Positive control expanders were sourced from current market suppliers.

Cloudz™ Treg CD3/CD28 Are Compatible with Different Serum Supplements

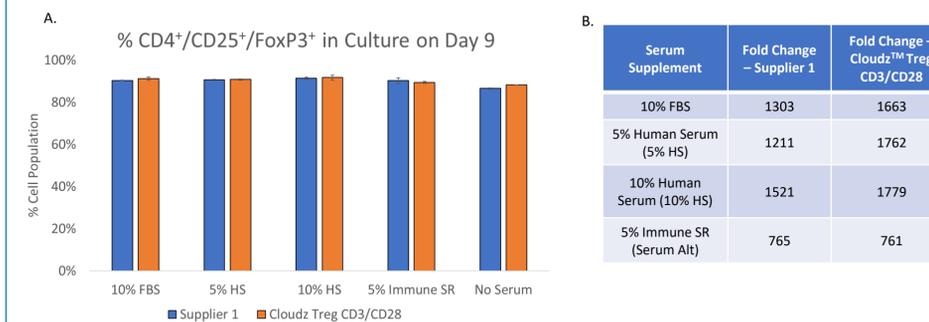


Figure 6. Treg cell growth and purity are similar across different serum-supplements. Treg purity on Day 9 was similar across different serum supplements and activation reagents (A). Cells stimulated with Cloudz™ Treg resulted in higher growth than Supplier 1 in all but the SR condition, in which both were similar (B). Data are presented as the mean ± S.D. (n=3).

CONCLUSION

- A working dose of 75 μL Cloudz™ Treg CD3/CD28 per 500,000 CD4⁺ cells leads to expansion of highly pure CD4⁺/CD25⁺/FoxP3⁺ cells.
- Cloudz™ Human Treg Expansion kit is compatible with:
 - Serum-free media expansion of Tregs.
 - A variety of serum supplements to support animal free cell therapy manufacturing
 - G-Rex6M cultureware, which allows for a scalable platform to manufacture Tregs to support immunotherapies.

FUTURE WORK

- Additional work is in progress to understand the suppression capability of Tregs expanded with Cloudz™ Treg CD3/CD28.
- Development is underway to deliver a GMP version of Cloudz™ Treg CD3/CD28 for GMP manufacturing of cell therapies.
- Optimizing protocols to support maximum expansion of Tregs with Cloudz™ Human Treg Expansion kit in a larger closed system culture vessel, G-Rex 100CS.