The benefits of supplementing CTS OpTmizer T Cell Expansion SFM, no phenol red

Introduction

Adoptive immunotherapy displays immense potential as an immunotherapeutic strategy for patients with advanced malignancies. Essential to this strategy is the *ex vivo* expansion of primary T cells obtained from the patient. Serum and phenol red have been mainstays as supplements in media used for T cell expansion. However, the inclusion of serum represents a significant hurdle to manufacturing of cell therapies due to limited supply, high cost, safety issues, batch-to-batch inconsistency, and the requirement for extensive testing, while phenol red may be associated with regulatory concerns and can interfere with sensors involved in automation.

Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free Medium (SFM), no phenol red, has proven to be a very effective complete, serum-free option for T cell expansion. However, there was speculation that additional supplementation could enhance the performance of this complete medium. We examined how the addition of Gibco™ CTS™ GlutaMAX™-I Supplement and CTS™ Immune Cell Serum Replacement (ICSR) to the complete medium influenced T cell growth, viability, and phenotype.

Materials and methods

T cell activation and expansion

T cells were activated in G-Rex™ vessels using Gibco™ Dynabeads™ Human T-Expander CD3/CD28 at a ratio of 3 beads for every cell and cultured in complete CTS OpTmizer T-Cell Expansion SFM, no phenol red, for 10 days in a Thermo Scientific™ Heracell™ 150i incubator set to 5% CO_2 and 37°C. Cells were initially seeded and activated at a density of 1 x 10^6 cells/mL and then left alone for 3 days. On day 3 and every 2–3 days after that, the density was maintained at 5 x 10^5 cells/mL. Additionally, 100 IU/mL of recombinant IL-2 was added on day 0 and every 2–3 days thereafter for the rest of the expansion phase. Growth and viability of cells cultured in complete medium or complete medium with supplements were measured using a Vi-CELL™ analyzer (Beckman Coulter) every 2–3 days, and the phenotype of the cells was assessed on day 10 using flow cytometry.

Phenotype analysis by flow cytometry

A total of 2 x 10^6 cells per donor were pelleted and stained with the following Invitrogen™ antibodies: Pacific Orange™ CD3, FITC CD4, Pacific Blue™ CD8, eBioscience™ APC CD62L, and eBioscience™ PE CCR7. Following staining, the cells were washed with PBS, pelleted by centrifugation (350 x *g* for 5 min), and fixed in 2% paraformaldehyde. Surface expression of the proteins was then assessed on the Gallios™ Flow Cytometer using Kaluza™ Analysis Software (Beckman Coulter).

Results

Primary T cells were assessed for growth, viability, and phenotype after culturing in CTS OpTmizer T Cell Expansion SFM, no phenol red, with or without additional supplements. The control was the complete medium, formulated as instructed in the product insert. Variations included the presence of 4 mM CTS GlutaMAX-I Supplement, 2.5% CTS ICSR, or both reagents. The growth curves in Figure 1A consistently show that cells supplemented with 2.5% CTS ICSR displayed increased growth. The viability results in Figure 1B illustrate that the growth-boosting ICSR can slightly lower viability. However, the addition of 4 mM CTS GlutaMAX-I Supplement on top

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of the 2.5% ICSR can reclaim some of the lost viability that occurs with ICSR alone. Cells cultured in medium containing both 2.5% CTS ICSR and 4 mM CTS GlutaMAX-I Supplement displayed increased growth with a smaller reduction in viability than in medium containing 2.5% CTS ICSR alone (Figure 1A, B).

The phenotypes of the expanded cells were assessed by evaluating the degree of differentiation as monitored by CCR7 and CD62L expression levels and quantifying the ratio of $CD8^+$ cells to $CD4^+$ cells. The graphs in Figures 1C and 1D report no consistent donor-independent phenotypic changes associated with the addition of CTS GlutaMAX-I Supplement, CTS ICSR, or the combination of the two reagents. As such, we can infer that the desirable central memory phenotype was maintained in the presence of these reagents as indicated by the consistent retention of CCR7 and CD62L expression (Figure 1C), and the ratio of $CD8^+$ cells to $CD4^+$ cells (Figure 1D).

Conclusions

We conducted a series of experiments to examine the effects of adding different reagents to CTS OpTmizer T Cell Expansion SFM, no phenol red. We report that the presence of 2.5% CTS ICSR enhances growth, and the simultaneous addition of 4 mM CTS GlutaMAX-I Supplement can help maintain viability during primary T cell expansion, while maintaining a desirable central memory phenotype.

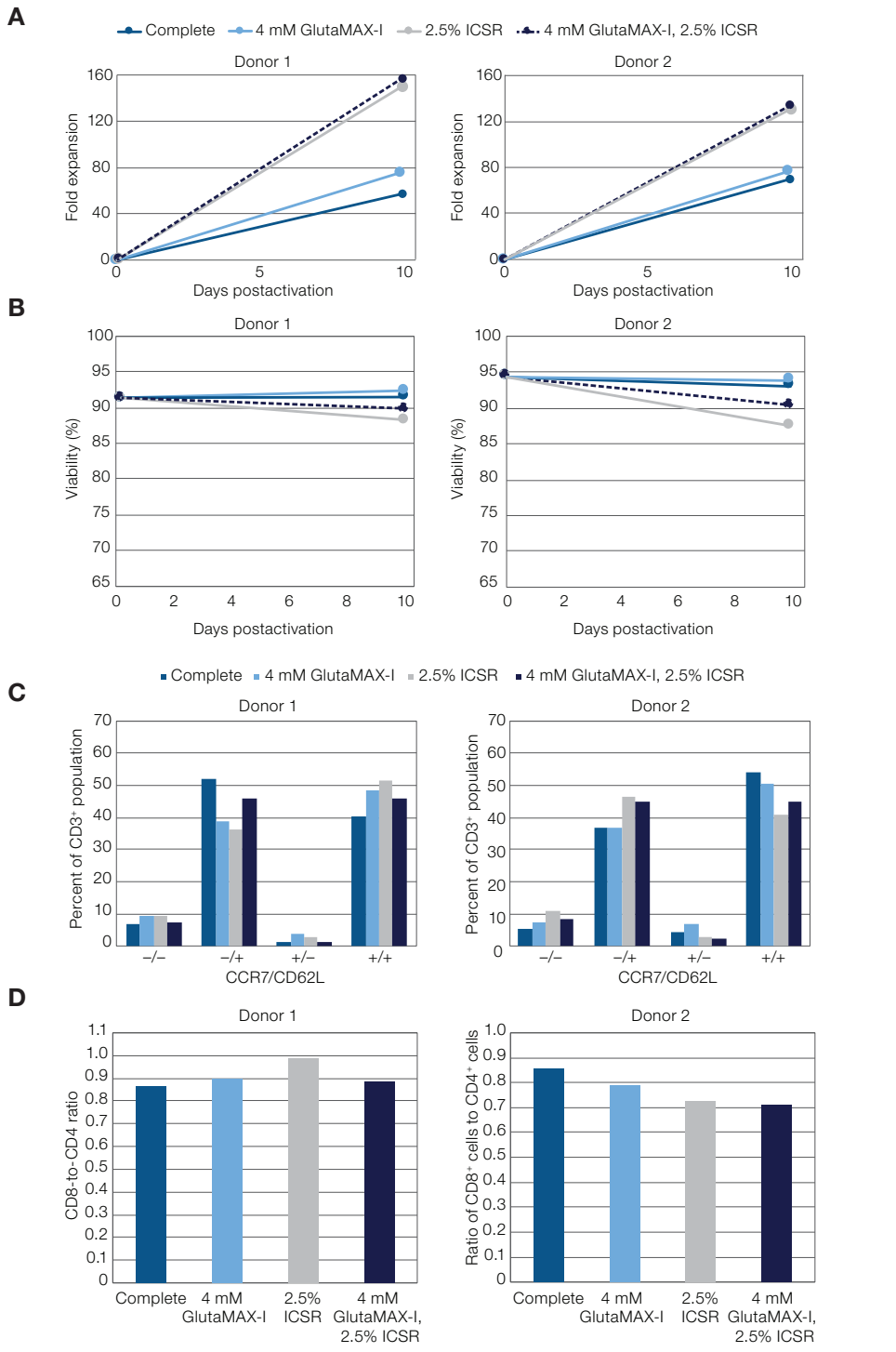

Figure 1. Analysis of primary T cell growth, viability, and phenotype. Cells were cultured in complete CTS OpTmizer T Cell Expansion SFM, no phenol red, with or without the indicated supplements. Data are shown for two independent T cell donors. **(A)** Cell growth was measured over time and reported as fold expansion. **(B)** Cell viability was monitored along with expansion of the cells. **(C)** The degree of differentiation was determined, as measured by CCR7 and CD62L expression within the $CD3^+$ population. **(D)** Phenotypic characterization was performed on day 10 to determine the ratio of $CD8^+$ cells to $CD4^+$ cells.

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