CTS OpTmizer T Cell Expansion SFM, no phenol red, displays T cell manufacturing workflow flexibility

Introduction

Chimeric antigen receptor (CAR) T cells have rapidly advanced from a promising preclinical immunotherapy to a commercially available treatment that has proven to be effective for various forms of leukemia and lymphoma. This success has driven an influx of companies into the immunotherapy landscape, with each company developing unique protocols for CAR T cell manufacturing. As variation in workflows and protocols increases, the demand for a T cell culture medium that is amenable to these processes becomes more challenging to satisfy. Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free Medium (SFM), no phenol red, from Thermo Fisher Scientific meets this challenge and demonstrates successful T cell expansion under a wide array of settings. Here we present results employing CTS OpTmizer T Cell Expansion SFM, no phenol red, in a variety of vessels ranging from static cell culture plates to a larger-scale rocking bioreactor, and determine that the medium expands T cells as effectively as a serum-containing medium.

Materials and methods

T cell isolation

Primary human T cells from healthy donors were negatively isolated from leukapheresis products.

Media

CTS OpTmizer T Cell Expansion SFM, no phenol red, and a basal medium from another supplier were used. This basal medium was supplemented with 5% human AB serum (hABs) (Gemini Bio-Products) and 2 mM Gibco™ L-Glutamine (Thermo Fisher Scientific). CTS OpTmizer T Cell Expansion SFM, no phenol red, was supplemented with 2 mM L-Glutamine and 2.5% of the included CTS OpTmizer T Cell Expansion Supplement; 4 mM Gibco™ CTS™ GlutaMAX™-I Supplement and 2.5% CTS™ Immune Cell Serum Replacement (ICSR) were also added.*

Seeding

For experiments in G-Rex™ vessels (Wilson Wolf Manufacturing), 5 x 10^6 T cells were seeded in 100 mL of the indicated medium. For experiments in plates and bags, T cells were seeded at 1 x 10^6 cells/mL in the indicated medium.

Activation and stimulation

For all experiments, T cells were activated with Gibco™ Dynabeads™ Human T-Expander CD3/CD28 (Thermo Fisher Scientific) at a ratio of 3 beads per T cell in the presence of 100 IU/mL of rIL-2 for experiments in plates and G-Rex vessels, and 300 IU/mL of rIL-2 for experiments in bags.

Static culture

For static plates (Fisher Scientific) and PermaLife™ bags (OriGen Biomedical), T cells were maintained at 5 x 10^5 cells/mL and counted on days 3, 5, 7, and 10 using a Vi-CELL™ analyzer (Beckman Coulter). For G-Rex vessels, medium was replaced on days 5 and 7. For all conditions, cultures were replenished with 100 IU/mL of rIL-2 on days 3, 5, and 7.

* Previous work has demonstrated that the addition of CTS GlutaMAX-I Supplement and CTS ICSR to CTS OpTmizer T Cell Expansion SFM can be beneficial to cell viability and growth, respectively.

Rocking bioreactor culture

Cells were activated in static PermaLife bags over days 0–3 as described. On day 3, the cells were inoculated into a 10 L Thermo Scientific™ Rocker BioProcess Container (BPC) on the Thermo Scientific™ HyPerforma™ Rocker Bioreactor. Cells were inoculated at a density of 0.25 x 10^6 cells/mL in 1,500 mL of the indicated expansion medium containing 100 IU/mL of rIL-2. The viable cell density was maintained at 0.25 x 10^6 cells/mL on days 5 and 7 up to a maximum volume of 5 L (rIL-2 was added with medium). Dissolved oxygen was maintained at 22% using automated gas control, and the pH was kept at or above 7 through the automated addition of base. Agitation speed and rocker angle were adjusted automatically throughout the run, based on the weight of the culture. Cell growth and viability were monitored daily, and phenotype was assessed at day 10 using flow cytometry.

Flow cytometry

Cellular phenotype was assessed on day 10 by staining T cells with the following Invitrogen™ antibodies: Pacific Orange™ CD3, FITC CD4, Pacific Blue™ CD8, eBioscience™ APC CD62L, and eBioscience™ PE CCR7 (Thermo Fisher Scientific).

Results

CTS OpTmizer SFM, no phenol red, is effective at expanding T cells in static plates

Traditionally, the most common vessels for T cell expansion and mammalian cell culture have been static culture plates or flasks. Figure 1 provides evidence that CTS OpTmizer T Cell Expansion SFM, no phenol red (supplemented with CTS ICSR and CTS GlutaMAX-I Supplement), fosters high levels of cell growth in static culture dishes, comparable to what is achieved with serum-containing medium from another supplier (Figure 1A). Moreover, the resultant cells exhibit a desirable phenotype, characterized by retention of the ratio of $CD8^+$ cells to $CD4^+$ cells (Figure 1B), an abundance of central memory cells, and a dearth of terminally differentiated cells, as measured by CD62L and CCR7 expression within the $CD3^+$ population (Figure 1C).

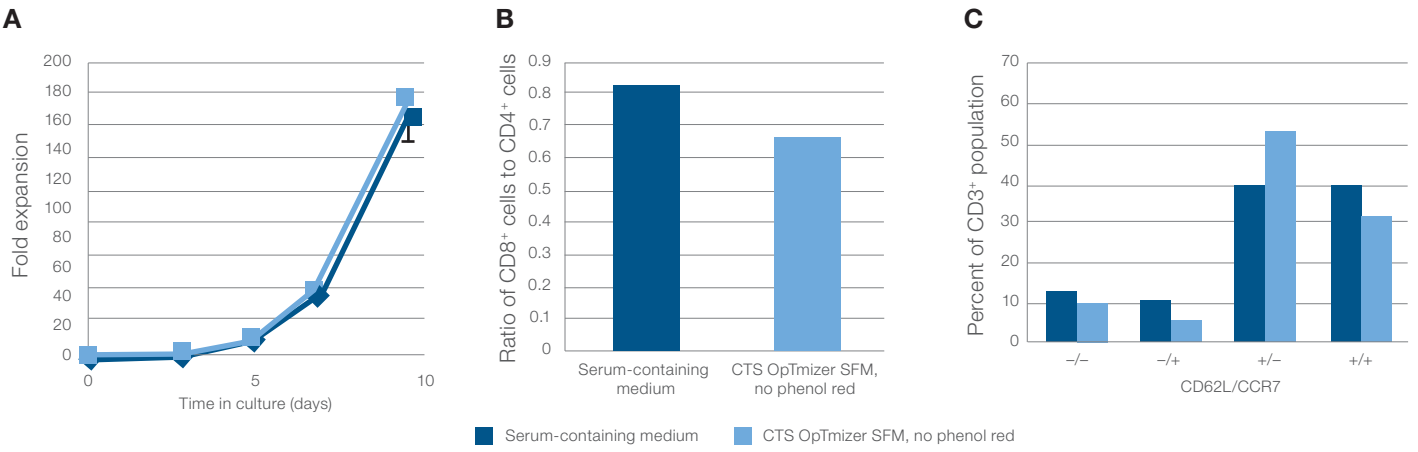

Figure 1. T cell expansion in static plates. T cells were cultured in CTS OpTmizer T Cell Expansion SFM, no phenol red, for 10 days in static cell culture plates. **(A)** Cell growth was measured over time and reported as fold expansion (error bars are the standard deviations of each data set). Phenotypic characterization was performed on day 10 to determine **(B)** the ratio of $CD8^+$ cells to $CD4^+$ cells and **(C)** the degree of differentiation, as measured by CD62L and CCR7 expression within the $CD3^+$ population. These investigations employed basal medium from another supplier containing 5% hABs, as a benchmark. Data represent the mean of three experimental replicates.

CTS OpTmizer SFM, no phenol red, is effective at expanding T cells in static culture bags

Another traditional vessel for T cell culture is static bags. Similar to the plates, we saw that T cell expansion in bags using CTS OpTmizer T Cell Expansion SFM, no phenol red (supplemented with CTS ICSR and CTS GlutaMAX-I Supplement), was again similar to the results from serum-containing culture methods (Figure 2A). The the ratio of $CD8^+$ cells to $CD4^+$ cells (Figure 2B) and differentiation status (Figure 2C) for the two media were also comparable to one another. There were good levels of both $CD8^+$ and $CD4^+$ cells and an appropriate distribution of cells toward the central memory end of the differentiation spectrum.

CTS OpTmizer SFM, no phenol red, is effective at expanding T cells in G-Rex vessels

More recently, the G-Rex (Gas-Permeable Rapid Expansion) culture platform has been a go-to static vessel for many scientists interested in T cell expansion. We tested the use of CTS OpTmizer T Cell Expansion SFM, no phenol red (supplemented with CTS ICSR and CTS GlutaMAX-I Supplement), in the G-Rex platform and found that it was able to support T cell growth up to levels on par with serum-containing medium (Figure 3A), and that the resultant population exhibited the expected ratio of $CD8^+$ cells to $CD4^+$ cells (Figure 3B) and high expression of markers associated with a central memory phenotype (Figure 3C).

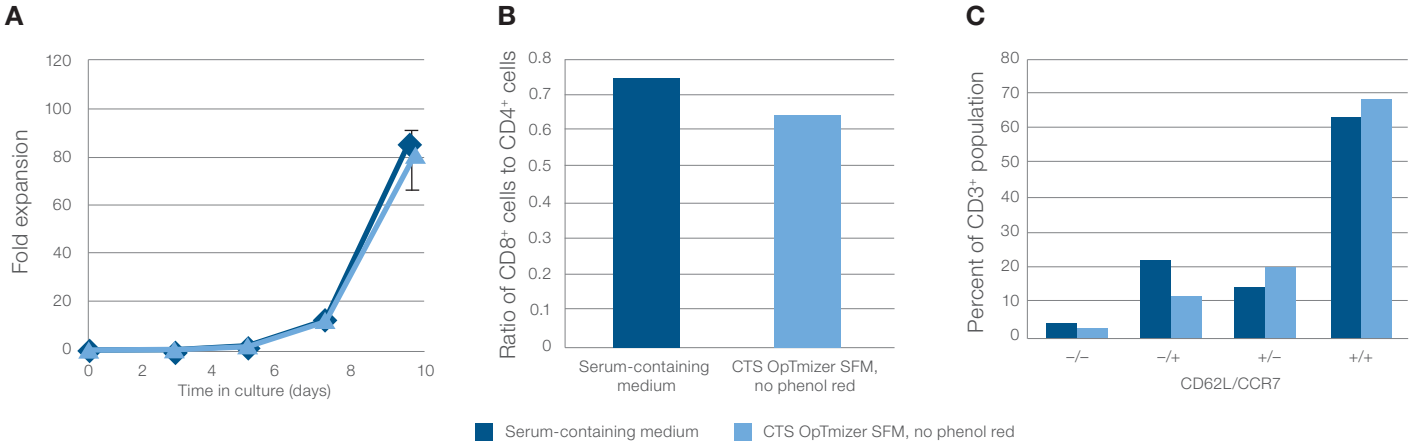

Figure 2. T cell expansion in static culture bags. T cells were cultured in CTS OpTmizer T Cell Expansion SFM, no phenol red, for 10 days in static cell culture bags. **(A)** Cell growth was measured over time and reported as fold expansion (error bars are the standard deviation of each data set). Phenotypic characterization was performed on day 10 to determine **(B)** the ratio of $CD8^+$ cells to $CD4^+$ cells and **(C)** the degree of differentiation, as measured by CD62L and CCR7 expression within the $CD3^+$ population. These investigations employed basal medium from another supplier containing 5% hABs as a benchmark. Data represent the mean of three experimental replicates.

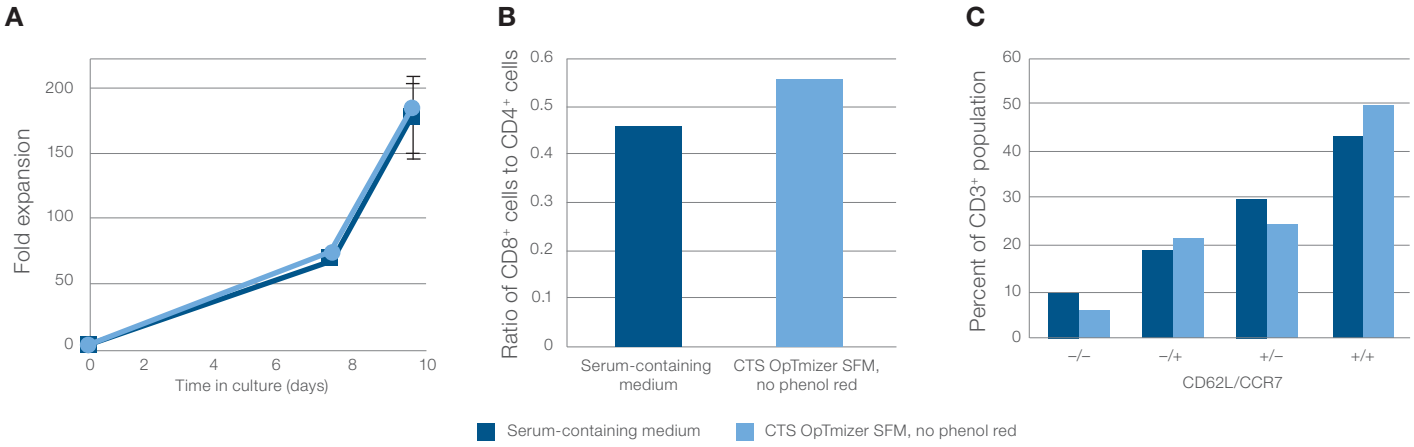

Figure 3. T cell expansion in G-Rex vessels. T cells were cultured in CTS OpTmizer T Cell Expansion SFM, no phenol red, for 10 days in 6-well G-Rex culture vessels. **(A)** Cell growth was measured over time and reported as fold expansion (error bars are the standard deviation of each data set). Phenotypic characterization was performed on day 10 to determine **(B)** the ratio of $CD8^+$ cells to $CD4^+$ cells and **(C)** the degree of differentiation, as measured by CD62L and CCR7 expression within the $CD3^+$ population. These investigations employed basal medium from another supplier containing 5% hABs as a benchmark. Data represent the mean of three experimental replicates.

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CTS OpTmizer SFM, no phenol red, is effective at expanding T cells in an automated rocking bioreactor

We also investigated the use of CTS OpTmizer T Cell Expansion SFM, no phenol red, in the HyPerforma Rocker Bioreactor, which is a large-capacity rocking bioreactor capable of automated control of gassing, feeding, and pH maintenance. Once again, CTS OpTmizer T Cell Expansion SFM, no phenol red (supplemented with CTS ICSR and CTS GlutaMAX-I Supplement), expanded cells as efficiently as the serum-containing medium (Figure 4A). Furthermore, these cells showed a ratio of $CD8^+$ cells to $CD4^+$ cells similar to that of the serum-containing medium (Figure 4B) and displayed a desirable distribution of differentiation markers, highlighted by a very low level of effector cells (Figure 4C).

Conclusions

CTS OpTmizer T Cell Expansion SFM, no phenol red, supports T cell expansion in a range of culture vessels with various workflows, including static culture plates, static culture bags, G-Rex vessels, and a rocking bioreactor. The resultant cell populations maintain an acceptable ratio of $CD8^+$ cells to $CD4^+$ cells, and the populations are consistently characterized by higher levels of cells expressing central memory markers and very few cells exhibiting a terminally differentiated phenotype.

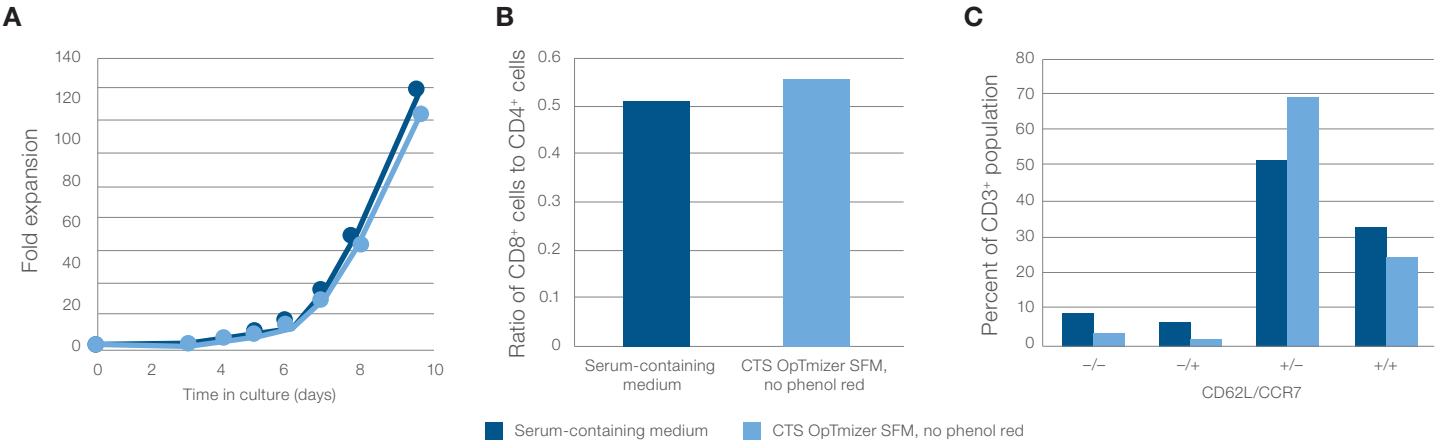

Figure 4. T cell expansion in the HyPerforma Rocker Bioreactor. T cells were cultured in CTS OpTmizer T Cell Expansion SFM, no phenol red, on the HyPerforma Rocker Bioreactor following a 3-day activation in a PermaLife static bag. DO and pH were maintained at 25% and ≥7.00, respectively. **(A)** Cell growth was measured over time and reported as fold expansion. **(B)** Phenotypic characterization was performed on day 10 to determine the ratio of $CD8^+$ cells to $CD4^+$ cells and **(C)** the degree of differentiation, as measured by CD62L and CCR7 expression within the $CD3^+$ population. These investigations employed basal medium from another supplier containing 5% hABs as a benchmark.

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