CTS™ Immune Cell SR for Serum Free Culture and Expansion of Human T cells

Sandy Kuligowski[2], Corey Smith[1], Grethe Økern[2], Tanja Aarvak[2], Rajiv Khanna[1], Karoline W. Schjetne[2]

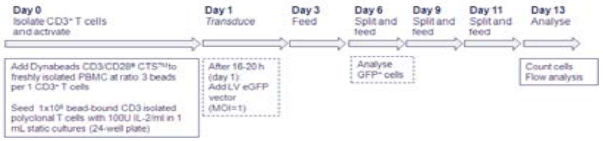
[1]QIMR Centre for Immunotherapy and Vaccine Development and Tumour Immunology Laboratory; QIMR Berghofer Medical Research Institute, Brisbane, Australia.
[2]Thermo Fisher Scientific, Ullernchausseen 52, N-0379 Oslo, Norway or Grand Island, NY

Introduction

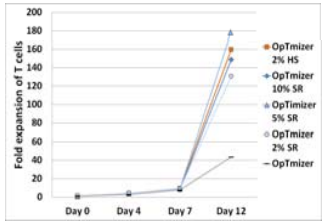
The manufacture of a majority of clinical T cell products for immunotherapy applications requires *in vitro* T cell culture and expansion. Commercialization of T cell manufacturing processes requires reagents that meet regulatory guidelines and ultimately help reduce manufacturing cost of goods. A key component in many T cell culture protocols, in addition to cell culture media and growth factors, is human serum. Human serum is expensive and requires extensive testing prior to use for manufacturing of a cGMP-compliant T cell product. To this end, we have tested a XenoFree serum replacement; CTS™ Immune Cell SR. CTS™ Immune Cell SR contains only defined components and can be used in combination with several different cell culture media to support *in vitro* culture and expansion of T cells.

Expansion of Dynabeads® CD3/CD28 CTS™ isolated and activated T cells

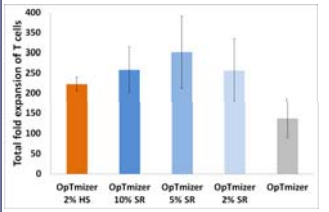
Methods:
- Polyclonal T cells from fresh PBMC were isolated and activated with Dynabeads® CD3/CD28 CTS™ and expanded for two weeks
- Cell culture media tested were CTS™ OpTmizer™ T cell Expansion SFM and X-VIVO™ 15 (not shown)
- Cell culture media were supplemented with either pooled AB human serum or CTS™ Immune Cell SR



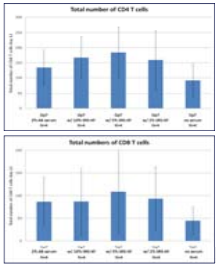
TOTAL FOLD EXPANSION



T cells expanded in OpTmizer™ supplemented with human serum or SR show similar growth kinetics and total fold expansion after 2 weeks in culture. Top panel: Growth kinetics from 1 representative donor. Bottom panel: Fold expansion of total T cells at the end of culture (day 12). Each bar represents an average of 4 blood donors.

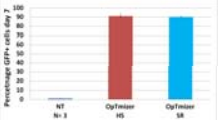


FOLD EXPANSION of CD4 and CD8 T CELLS



CTS™ Immune Cells SR support expansion of both CD4 and CD8 T cells subsets

SUPPORT LENTIVIRAL TRANSDUCTION



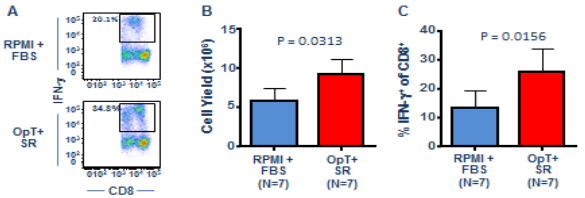
CTS™ Immune Cells SR supports transduction of T cells using a lentiviral vector expressing GFP

ACKNOWLEGDEMENT
We thank James L. Riley and Andrew Medvec from the University of Pennsylvania, Department of Microbiology for providing the pELNS-GFP lentiviral vector

Expansion of virus-specific T cells

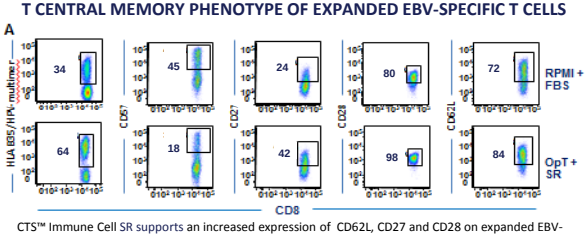
Methods:
- PBMC from healthy CMV seropositive donors were cultured with autologous PBMC pulsed with a pool of CMV-encoded CD8+ T cell peptides for 14 days
- Cell culture media tested were RPMI with FBS or CTS™ OpTmizer™ T cell Expansion SFM with CTS™ Immune Cell SR. Both media were supplemented with 120 U/ml IL-2 from day 3 and then every 3-4 days
- T cell specificity was determined using an intracellular IFN-γ assay following recall with a pool of defined CMV-encoded, CD8+ T cell peptide epitopes.

INCREASED CELL YIELD AND CYTOKNE SECRETION OF CMV-SPECIFIC T CELLS



T cell yield (B) and interferon-γ secretion (A,C) from CMV-stimulated T cells is increased when cells were cultured in OpTmizer media supplemented with CTS™ Immune Cells SR.

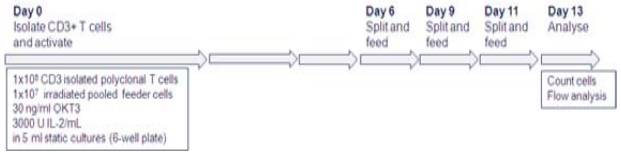
T CENTRAL MEMORY PHENOTYPE OF EXPANDED EBV-SPECIFIC T CELLS



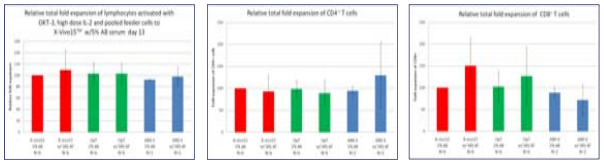
CTS™ Immune Cell SR supports an increased expression of CD62L, CD27 and CD28 on expanded EBV-specific T cells which indicated a T central memory phenotype. CD57 expression is decreased.

Expansion of OKT3-activatedd T cells

Methods:
- Polyclonal T cells were negatively isolated from fresh PBMC, activated *in vitro* with OKT3 mAb, irradiated pooled feeder cells and high dose IL-2 and expanded for two weeks
- Cell culture media tested were CTS™ OpTmizer™ T cell Expansion SFM , X-VIVO™ 15 or CTS™ AIM-V® Medium
- Cell culture media were supplemented with either pooled AB human serum or 10% CTS™ Immune Cell SR



TOTAL FOLD EXPANSION | FOLD EXPANSION of CD4 T cells | FOLD EXPANSION of CD8 T cells



T cells expanded in cultures supplemented with pooled human serum or CTS™ Immune Cell SR show similar growth kinetics and total fold expansion after 2 weeks in culture. Both CD4+ and CD8+ T cell subsets are expanded.

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Conclusions

- CTS™ Immune Cell SR supports expansion of Dynabeads® CD3/CD28 CTS™-activated polyclonal T cells and virus-specific T cells in combination with several commonly used cell culture media
- CTS™ Immune Cell SR supports transduction and expansion of gene-modified T cells
- CTS™ Immune Cell SR is xeno-free and contains only fully tested human-derived or human recombinant proteins which facilitates supply security for clinical large scale and commercial therapies
- CTS™ Immune Cell SR facilitates expansion of T cells with T central memory phenotype