A CHEMICALLY-DEFINED, ANIMAL COMPONENT-FREE *EX VIVO* EXPANSION PROCESS FOR ACTIVATED HUMAN T CELLS

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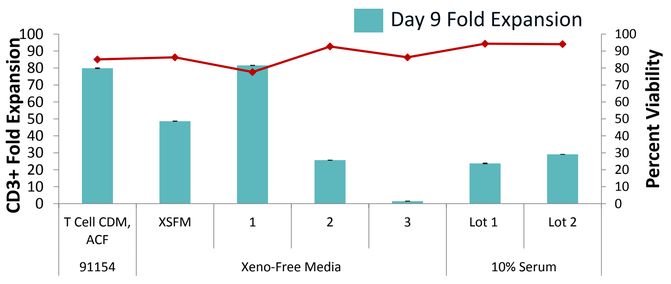
Introduction

T cell based immunotherapy applications have recently drawn great interest and notoriety due to their clinical potential as the next-generation of life-saving therapies for cancer patients. Generation of desired T cell populations consistently and efficiently is essential for the successful development of T cell based immunotherapy, and this requires the establishment of a scalable and robust *ex vivo* manufacturing process. However, key components in culture media often include serum or human albumin-containing undefined components which pose as a threat of introducing adventitious pathogens. Presented in this study is the development of a chemically-defined (CD), animal-component-free (ACF) T cell basal culture medium that is scalable and suitable for T cell therapy products. In addition to its utility in static culture conditions using T-flasks, the CD, ACF basal medium supports the rapid expansion of T cells with high viability in other commonly used expansion vehicles including cell culture bags and G-Rex® cell culture device. The expanded cells may also be cryopreserved and reactivated for future use.

Methods

T cells derived or purified from human peripheral blood mononuclear cells (PBMCs) were activated using anti-human $CD3^+$ and CD28 antibodies. Cells were inoculated at 1 – 2.5 $x10^5$ cells/mL and fed every two to three days with fresh basal medium supplemented with IL-2 at 200U/mL. For T cell polarization, cells were treated with different cytokines and antibodies as indicated.

Results



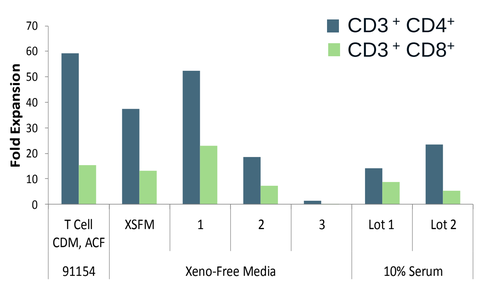
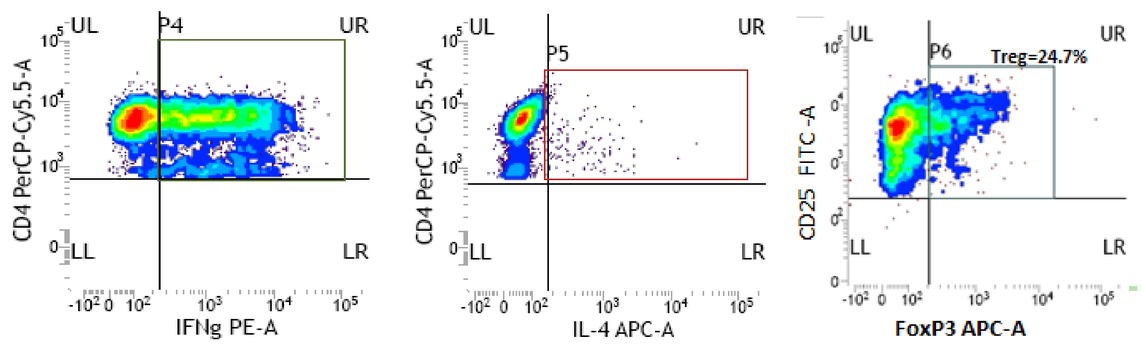
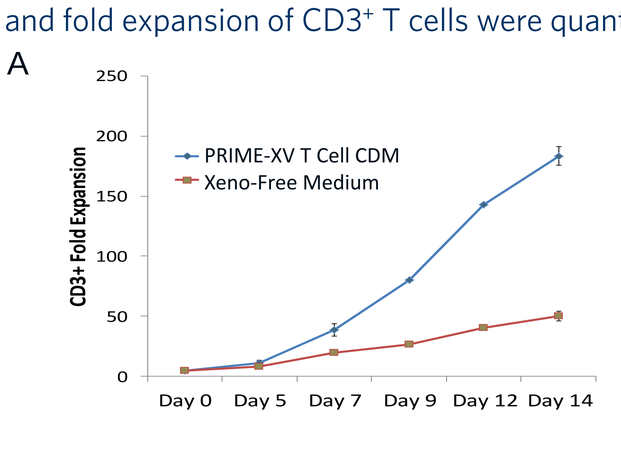


Figure 1. PRIME-XV® T Cell CDM Expansion of T Cells Compared to Xeno-Free or Serum-Containing Media. Human peripheral blood mononuclear cells, seeded at $1x10^5$ cells/mL, were activated and cultured in PRIME-XV T Cell CDM or commercially available xeno-free expansion media supplemented with IL-2 . After 9 days, viability and fold expansion of $CD3^+$ T cells were quantified.





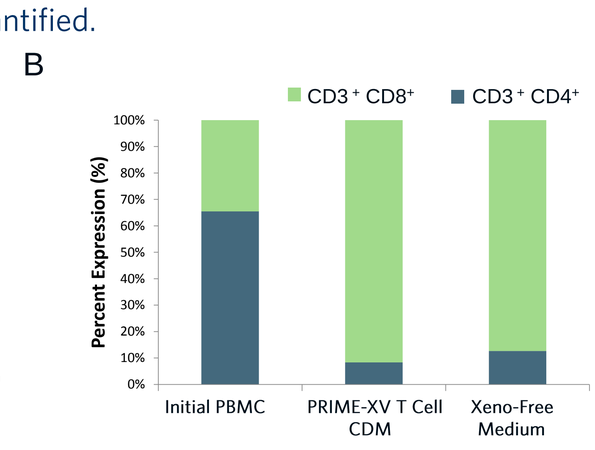


Figure 2. PRIME-XV T Cell CDM Expansion of T Cells in Cell Culture Bags. Gas-permeable cell culture bags were coated with anti-human $CD3^+$ antibody (clone OKT3) at 2-8°C overnight. Media supplemented with IL-2 at 200IU/mL were added with PBMCs and cultured for 14 days. $CD3^+$ T cell fold expansion was quantified on days 5, 7, 9, 12,14 (A) and flow cytometry analysis was performed to compare the expression of $CD3^+$ $CD4^+$ /$CD3^+$ $CD8^+$ (B)

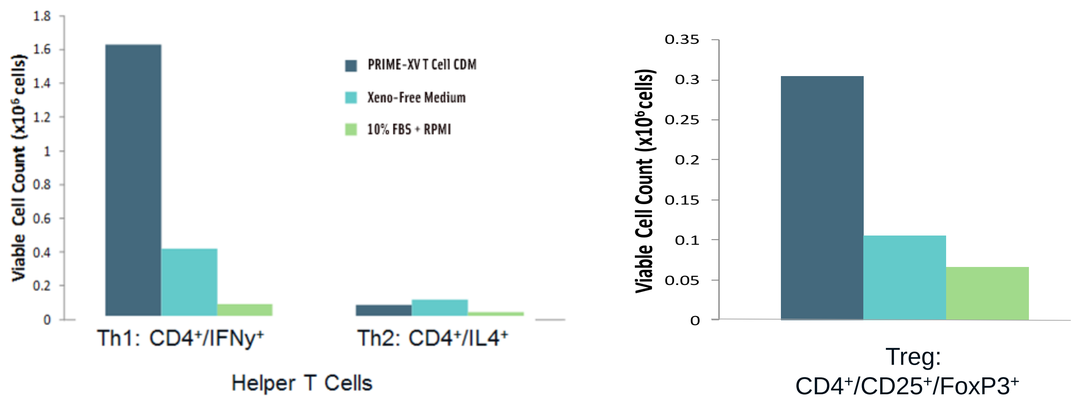


Figure 4. PRIME-XV T Cell CDM Enhances the Polarization of $CD4^+$ T Cell to T Regulatory Cells. Purified $CD3^+$ T cells were activated and expanded for two days in media containing IL-2. For the polarization of Th_1 cells, T cells were cultured for 72 hours in media containing 5ng/mL of recombinant human IL-12 and 10µg/mL of anti-human IL-4 antibodies. For T regulatory cell polarization, T cells were cultured for 72 hours with 50ng/mL of recombinant TGF-β1 and 6ng/mL of retinoic acid. Flow cytometry analysis showed representative populations of Th_1, Th_2, and T regulatory cells.

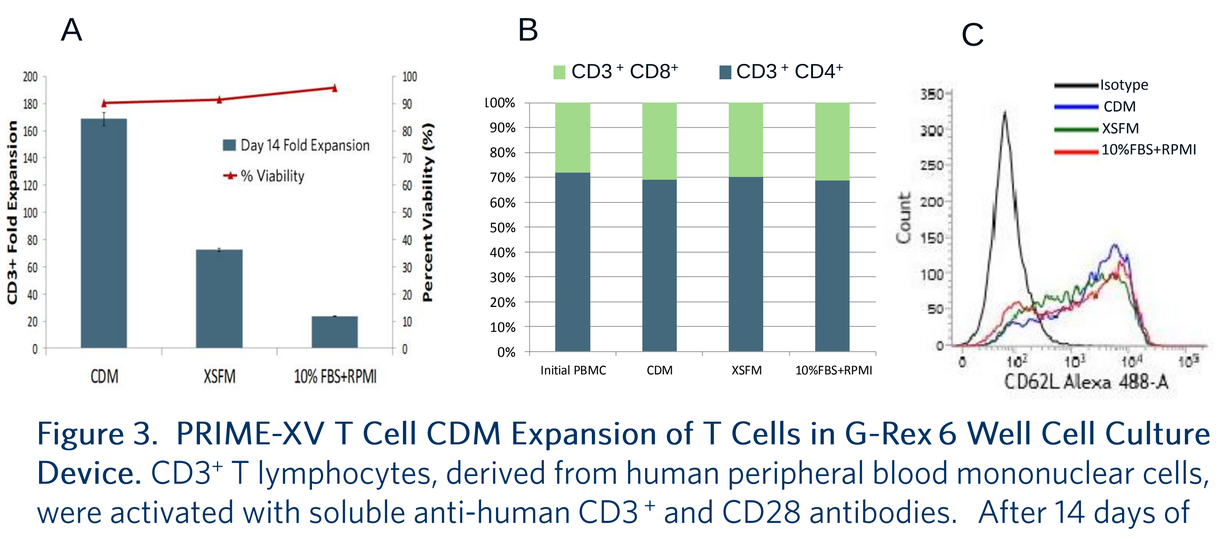


Figure 3. PRIME-XV T Cell CDM Expansion of T Cells in G-Rex 6 Well Cell Culture Device. $CD3^+$ T lymphocytes, derived from human peripheral blood mononuclear cells, were activated with soluble anti-human $CD3^+$ and CD28 antibodies. After 14 days of culture in various media supplemented with IL-2, cells were harvested and analyzed for viability and fold expansion (A). Flow cytometry analysis was performed to compare the expression of $CD3^+$ $CD4^+$ /$CD3^+$ $CD8^+$ (B) and CD62L (C) in initial PBMC population to T cells cultured in various media conditions.

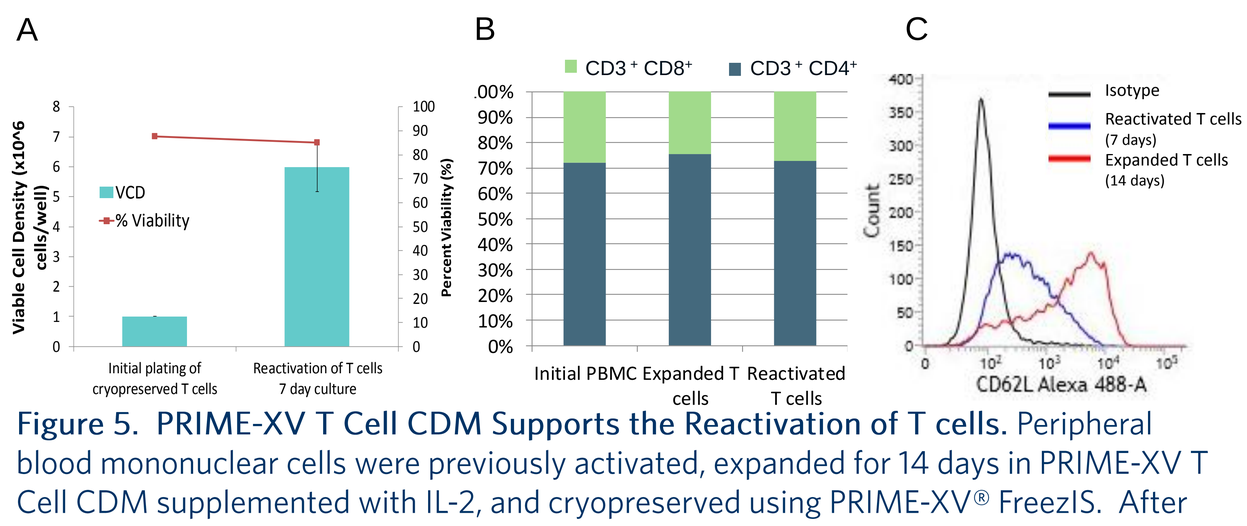


Figure 5. PRIME-XV T Cell CDM Supports the Reactivation of T cells. Peripheral blood mononuclear cells were previously activated, expanded for 14 days in PRIME-XV T Cell CDM supplemented with IL-2, and cryopreserved using PRIME-XV® FreezIS. After one week in liquid nitrogen, cells were thawed and reactivated for expansion. Growth and viability of expanded cryopreserved cells were quantified (A). Flow cytometry analysis was performed to compare the expression of $CD3^+$ $CD4^+$ /$CD3^+$ $CD8^+$ (B) and CD62L (C) in initial PBMC population to expanded or reactivated cells grown in IL-2 supplemented PRIME-XV T Cell CDM.

Conclusion

PRIME-XV T Cell CDM supports the rapid expansion of T cells while maintaining high viability compared to other xeno-free or serum-containing media. This study demonstrates the ability for large scale expansion of T cells in cell culture bags and G-Rex cell culture device, and expanded cells show an enhanced polarization potential into the desired T cell subsets. Overall, this is the first CD, ACF, cGMP-manufactured T cell expansion medium developed to provide the quality and consistency necessary for T cell based immunotherapy applications.