Poster 916
ISCT 2025, New Orleans

Serum Free and Feeder Free Media System for Expansion and Differentiation of Primary Human B Cells for Cell and Gene Therapy

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Abstract

Background and Aims: Advances in gene editing and cell culture systems are driving the development of novel cell and gene therapies. A recent example of a novel cell therapy platform is the use of engineered B cell derivatives for diverse clinical applications. Unlike traditional gene therapies, B cell-based therapies present the potential of being re-dosable, without the need for myeloablative pre-conditioning. Adoptive cell therapies using engineered B cell products as *in vivo* "drug factories" for long term generation of biologics such as broadly neutralizing antibodies and therapeutic proteins are currently under investigation[1]. B cells spontaneously differentiate and die during *in vitro* expansion. Similarly, *in vitro* differentiation of B cells generates plasmablasts (PBs) and plasma cells (PCs) with low viability. For successful B cell-based therapies, cell culture workflows that i) reliably expand B cells without spontaneous differentiation and ii) promote directed differentiation of B cells to produce viable PBs and PCs are desired. Cell therapy systems (CTS™) AIM-V is a serum-free medium and CTS™ Immune Cell Serum Replacement (ICSR) is a defined xeno-free supplement that has been widely used in clinical trials for the culture of various immune cells. In this study, we confirm the suitability of CTS™ AIM-V and CTS™ ICSR for the expansion and differentiation of B cells.
Methodology: B cells were expanded for 14 days in CTS™ AIM-V supplemented with CTS™ ICSR. B cells were also differentiated to antibody secreting cells (ASCs) by a tri-step protocol involving 7 days of expansion followed by directed differentiation to PBs and then PCs for 6 more days.
Results: B cells expanded in CTS™ AIM-V medium supplemented with CTS™ ICSR exhibited greater than 100-fold expansion, shifting from naïve to memory phenotype with minimal differentiation to PBs and PCs. The expansion was three times greater, and spontaneous differentiation was significantly lower than other commercially available B cell media. B cells differentiated in CTS™ AIM-V supplemented with CTS™ ICSR and a proprietary supplement showed robust differentiation generating greater than 10 times the number of live ASCs on day 13 and was significantly greater than other commercially available B cell media. These differentiated B cells secreted IgG as confirmed by ELISA.
Conclusion: In conclusion, we demonstrate the use of CTS™ AIM-V and CTS™ ICSR as a reliable serum free media system for the expansion and differentiation of B cells.

Introduction

B cells are important players in humoral immunity. They are being investigated for cell-based therapies because of their ease of isolation from peripheral blood, ability to expand *in vitro*, action as antigen presenting cells, inherent function to produce large quantities of antibodies, and their prolonged longevity *in vivo*.

However, *ex vivo* expansion and differentiation procedures for B cells are not efficient or standardized in the field like T cells. Different culture systems produce different populations of B cells that complicate their use as efficient cell therapies. In this study, we demonstrate the use of CTS™ AIM-V and CTS™ ICSR for feeder free expansion and differentiation of B cells.

Methods

Peripheral blood B cells were cultured in either CTS™ AIM-V medium supplemented with CTS™ ICSR with or without a proprietary chemically defined supplement (CDS), a serum free B cell expansion media kit from another supplier, and IMDM medium supplemented with 10% fetal bovine serum (FBS), a commonly used medium for B cell culture. Cellular counts for expansion and differentiation were recorded on an automated cell counter and cell phenotype was analyzed on the Attune CytPix flow cytometer. Human IgG was analyzed in cell supernatants by ELISA. Data is represented as mean ± standard error of mean.

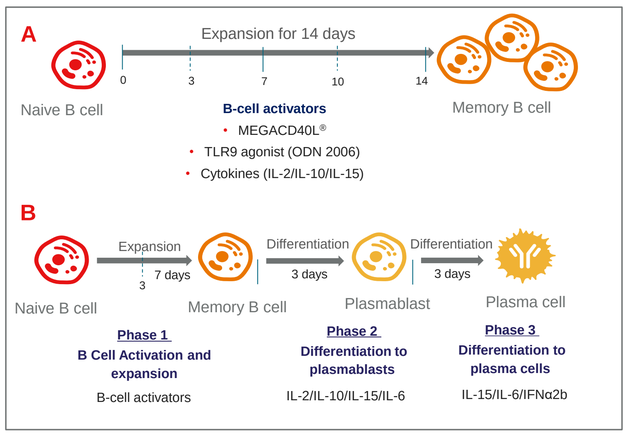


Fig. 1. Workflow for B cell expansion (A) and B cell differentiation to ASC (B). Vertical dotted lines indicate fresh media top up while solid lines indicate full media change with activators & cytokines.

Results

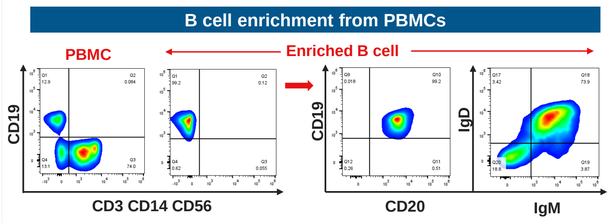


Fig. 2. B cells are enriched from PBMCs using Dynabeads™ Untouched™ Human B Cells Kit. Enriched B cells (CD19⁺CD20⁺) are positive for IgD and IgM indicating mostly naïve B cells.

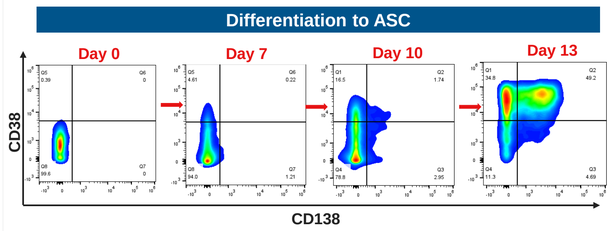


Fig. 5. B cells cultured in AIM-V and ICSR can be directed to differentiate to plasmablasts (CD38⁺CD138⁻) and plasma cells (CD38⁺CD138⁺) by a tri-step protocol.

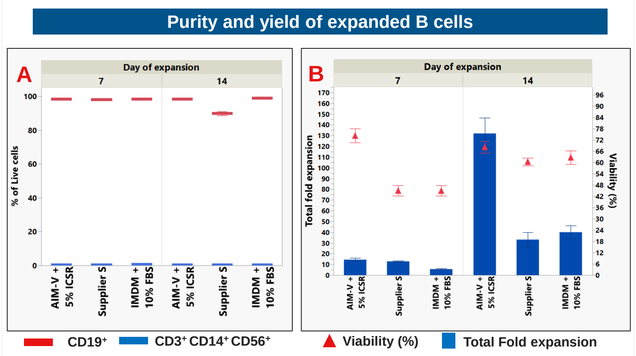


Fig. 3. B cells expanded in AIM-V + 5% ICSR for 14 days maintain purity (A) and show significantly increased fold expansion than other serum free supplier and IMDM + 10% FBS for B cell culture (B). N=3 donors.

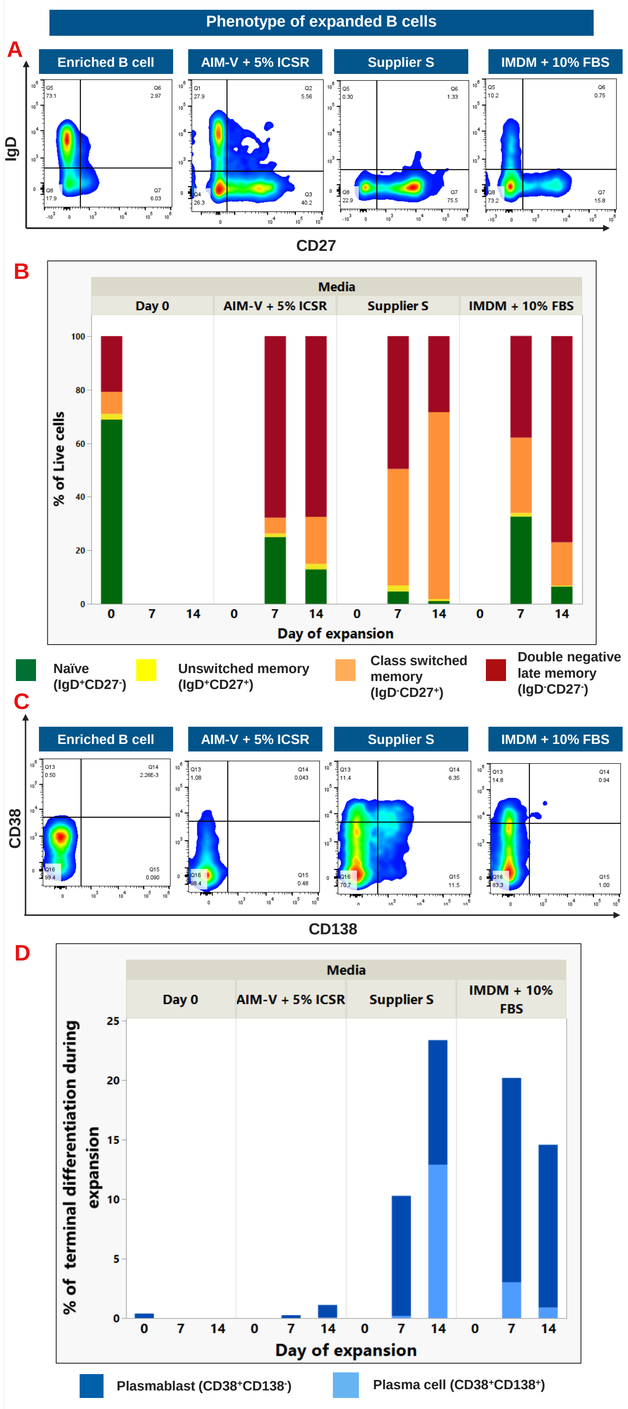
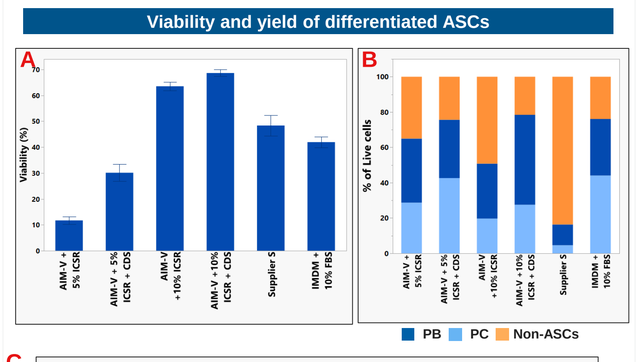


Fig. 4. B cells undergoing expansion for 14 days change phenotype from predominantly naïve to memory B cells (A&B). B cells expanded in AIM-V + 5% ICSR also maintain more IgD expression (naïve marker) (A&B) and show minimal terminal differentiation to plasmablasts and plasma cells (C&D) during expansion. N=3 donors.



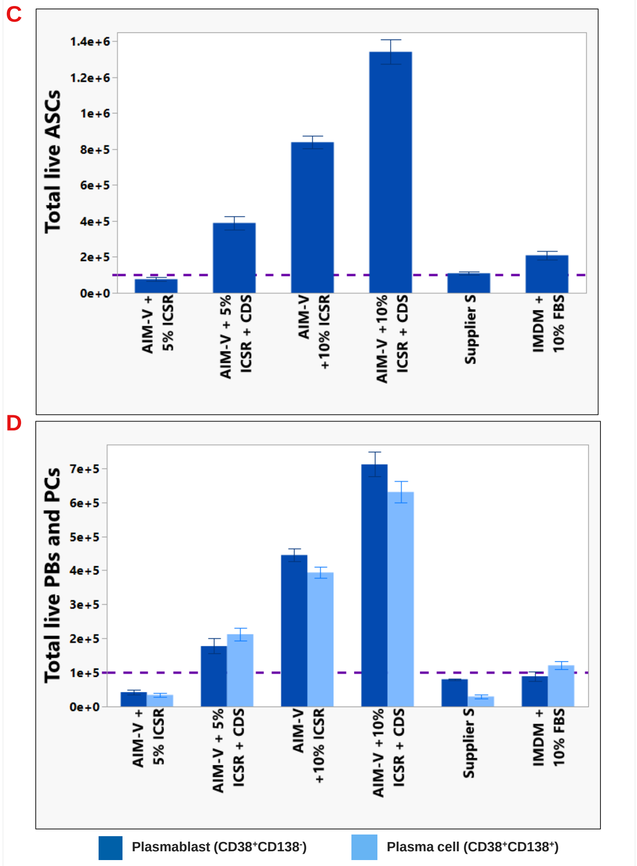


Fig. 6. ASC viability (A) and yield is significantly increased by supplementing AIM-V medium with 10% ICSR and a chemically defined supplement (CDS) as compared to other serum free supplier and IMDM + 10% FBS (B-D). Purple dotted lines indicate initial seeding counts of B cells on Day 0 of differentiation. N = 1-3 donors.

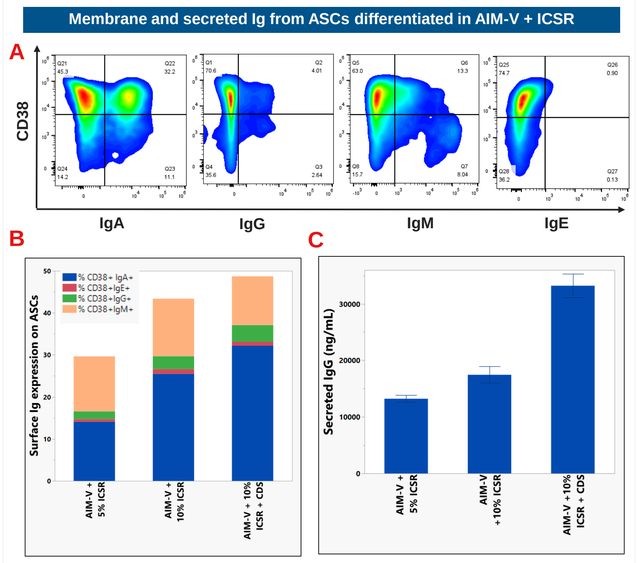


Fig. 7. Addition of a chemically defined supplement and 10% ICSR to AIM-V increase membrane expression of IgA and IgG (A&B) and increase levels of secreted IgG as evidenced by IgG ELISA of ASC supernatants collected at the end of differentiation (C).

Conclusions

- B cells can be reliably isolated from PBMCs by Dynabeads™ Untouched™ Human B Cells Kit with ~ 99% purity.
- B cells can be expanded in a serum free and feeder free format with CTS™ AIM-V supplemented with CTS™ ICSR using a well described protocol in literature[1]. The cumulative fold expansion of viable B cells cultured in CTS™ AIM-V supplemented with CTS™ ICSR was significantly greater than other commonly used B cell media. There was more retention of naïve markers and minimal terminal differentiation of B cells during expansion. This is beneficial to researchers who are looking to only expand B cells and do not want B cells to terminally differentiate and die during the expansion process.
- B cells can be differentiated to antibody secreting cells by a tri-step protocol as described in literature[1]. Supplementation of CTS™ AIM-V with 10% ICSR, and a chemically defined supplement significantly boost ASC yield. Differentiated ASCs are functional in producing IgG. This is beneficial to researchers that want directed differentiation of B cells to plasmablasts and plasma cells.
- CTS™ AIM-V and CTS™ ICSR are widely used in clinical trials for the culture of immune cells like T cells and myeloid cells. Depending on the need of researchers, CTS™ AIM-V and CTS™ ICSR can be tailored to selectively expand or differentiate B cells for various clinical applications.

References

1. Hung KL, Meitlis I, Hale M, Chen CY, Singh S, Jackson SW, Miao CH, Khan IF, Rawlings DJ, James RG. Engineering Protein-Secreting Plasma Cells by Homology-Directed Repair in Primary Human B Cells. Mol Ther. 2018 Feb 7;26(2):456-467. doi10.1016/j.ymthe.2017.11.012. Epub 2017 Nov 22. PMID: 29273498; PMCID: PMC5835153.

Product information

- Extensive document package for regulatory support
- Closed system compatible. Constructed from Aegis 5-14 film and includes sterile, weldable DEHP-free PVC tubing with Luer lock and C-Flex tubing with MPC quick connector.
- Innovative packaging for frozen supplements

Product	Format	Catalog #
CTS™ AIM-V™ Medium	1 L bottle	A3830801
	2 L bag	A4672701
	10 L bag	A3830802
CTS™ Immune Cell Serum Replacement (ICSR)	50 mL bottle	A2596101
	250 mL bag	A4702901
	500 mL bottle	A2596102
	1 L bag	A4702902

*Animal origin free recombinant cytokines were from Gibco™ PeproTech™

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