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CTS™ AIM-V™ Serum Free Medium for Dendritic Cell Culture

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ABSTRACT

Dendritic cells (DCs) are unique antigen presenting cells that act as a bridge between the innate and adaptive immune system. Upon sensing foreign pathogens or endogenous danger signals, DCs upregulate co-stimulatory molecules, produce cytokines, uptake, and process antigen, migrate to lymph nodes, and present antigen to T-cells to mount an immune response. In the current cell therapy landscape, tumor antigen pulsed autologous DCs are widely used in cancer immunotherapy to augment T-cell mediated tumor killing.

DCs are commonly derived by *ex vivo* differentiation from autologous monocytes and have historically been cultured in RPMI based media containing fetal bovine or human serum. Since serum media are the current gold standards for cell therapy applications, newer cell therapy compatible media systems need to be validated for myeloid cell therapies.

Here, we validate the use of CTS AIM-V, a serum free, closed system compatible medium to culture functional DCs from peripheral blood monocytes. Untouched monocytes were enriched from PBMCs and cultured in CTS AIM-V with recombinant animal origin free IL-4 and GM-CSF, followed by antigen challenge and maturation with various maturation cocktails. Mature DCs differentiated in CTS AIM-V medium showed equivalent or better yield as compared to other suppliers and robustly expressed DC markers like CD83 and CD11c, B7 family of co-stimulatory molecules, chemokine receptor CCR7, and upregulation of the MHC class II cell surface receptor HLA-DR. DCs matured with toll like receptor-4 agonists and interferon gamma secreted high levels of the bioactive heterodimer of interleukin 12 that drives Th1 polarization in T-cells. Mature DCs exhibited great functionality in inducing allogeneic T-cell proliferation and activation. Similarly, MHC-I restricted antigen peptide pulsed DCs induced autologous cytotoxic T-cell proliferation and activation indicating robust performance in antigen presentation. These functions were retained after cryopreservation confirming the suitability of CTS AIM-V medium for culture of clinically relevant DCs. In conclusion, we demonstrate the use of CTS AIM-V medium as a reliable medium for ex-vivo differentiation of monocyte derived DCs for myeloid cell therapies.

INTRODUCTION

Serum free media are preferred for cell therapy applications and help minimize batch to batch product variability and improve reproducibility of clinical studies. CTS AIM-V is a serum free medium widely used for multiple immune cell cultures[1] and available in several formats for both research and manufacturing applications. CTS Immune Cell Serum Replacement (ICSR) is a defined xeno-free supplement that has been widely used in serum free T-cell cultures. Recently, myeloid cells have gained interest as attractive candidates for therapies against solid tumors. Among many other myeloid cell types, monocyte derived DCs are widely used in cancer immunotherapies and as cancer vaccines[1]. Here, we demonstrate that CTS AIM-V serum free medium with or without CTS ICSR supplementation can be used to culture functional DCs.

METHODS

Untouched CD14+ monocytes (5x10[5] cells per well) were seeded in Nunc™ Delta Surface 12 well plates and cultured in CTS AIM-V±5% ICSR or other supplier's media with 500 IU/mL IL-4 and 1000 IU/mL GM-CSF for 7 days with fresh media supplementation on days 3 and 5. On Day 5, i) a traditional maturation cocktail first described by Jonuleit *et al* [2], consisting of 10 ng/mL tumor necrosis alpha, 10 ng/mL interleukin 1 beta, 15 ng/mL interleukin 6, and 1 µg/mL prostaglandin E2; or ii) an alternative maturation cocktail consisting of a Toll like receptor 4 agonist, monophosphoryl lipid A (MPLA) (5 µg/mL), and 2000 IU interferon gamma (IFNg)[3] was added in some samples to obtain mature DCs (mDCs). In some experiments, immature DCs (iDCs) were pulsed with 2 µg/mL viral antigen peptide mix (CEF) before addition of maturation agents. mDCs and iDCs were harvested on Day 7 and analyzed for DC yield, phenotypic characterization and interleukin 12 secretion. mDCs and iDCs were co-cultured with allogeneic or autologous T-cells at a ratio of 1:20 for proliferation assays, and ~1:10 for activation assays. T-cell proliferation was recorded in cell proliferation dye-stained T-cells on day 4 of co-culture. T-cell activation was analyzed by intracellular labeling of T-cells on day 5 of co-culture.

Cell count and viability was analyzed by a Vi-Cell Blu automated cell counter. Brightfield and immunofluorescent imaging of monocytes, iDCs and mDCs were performed under an EVOS M7000 imaging system. Assessment of surface and intercellular markers, and cell proliferation were performed on an Invitrogen Attune CytPix Flow Cytometer and analyzed by Flow Jo software v10.

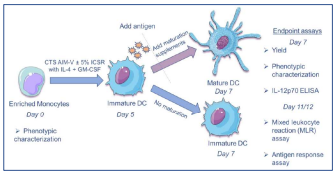

Fig. 1. Workflow for culturing functional monocyte derived DCs using CTS AIM-V medium. Cell images were obtained from https://smart.servier.com/ and modified under Creative Commons Legal Code.

RESULTS

Monocyte isolation from PBMCs

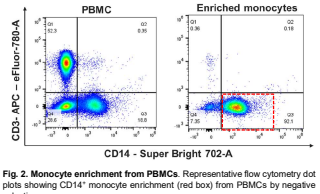

Fig. 2. Monocyte enrichment from PBMCs. Representative flow cytometry dot plots showing CD14+ monocyte enrichment (red box) from PBMCs by negative selection.

Yield of monocyte derived DCs

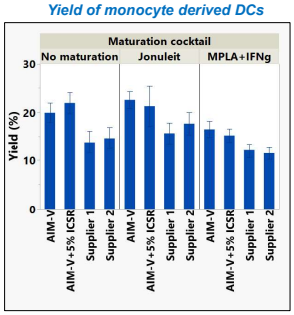

Fig. 3. Percentage yield of DCs cultured in various serum free media and matured with different maturation agents. Percentage yield was calculated by dividing number of viable DCs on day 7 by number of viable monocytes seeded on day 0, multiplied by 100. CTS AIM-V with or without 5% ICSR showed comparable or better yield than other supplier media for both mDC and iDC. Data was pooled from 6-10 donors and represented as Mean ± Standard error of mean.

Morphology of DCs cultured in CTS AIM-V

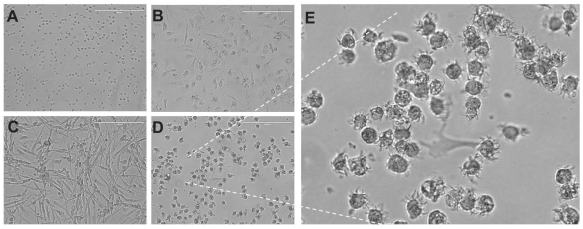

Fig. 4. Morphology of monocytes, immature and mature DCs cultured in CTS AIM-V. A) Monocytes on Day 0, B) iDC, C) mDC (MPLA + IFNg), D) mDC (Jonuleit), E) enlarged image of mDC (Jonuleit) on Day 7. Scale bar in A-D corresponds to 200 µm. Monocytes are smaller and round cells, iDCs exhibited enlarged flattened adherent morphology with some loosely adherent cells. On maturation with the Jonuleit's cocktail, mDCs differentiated in CTS AIM-V were round, loosely adherent and showed dendritic processes. On the other hand, alternative cocktails like MPLA + IFNg generated mDCs that were elongated in shape and were more firmly adherent.

Phenotypic characterization of monocytes, iDC and mDC

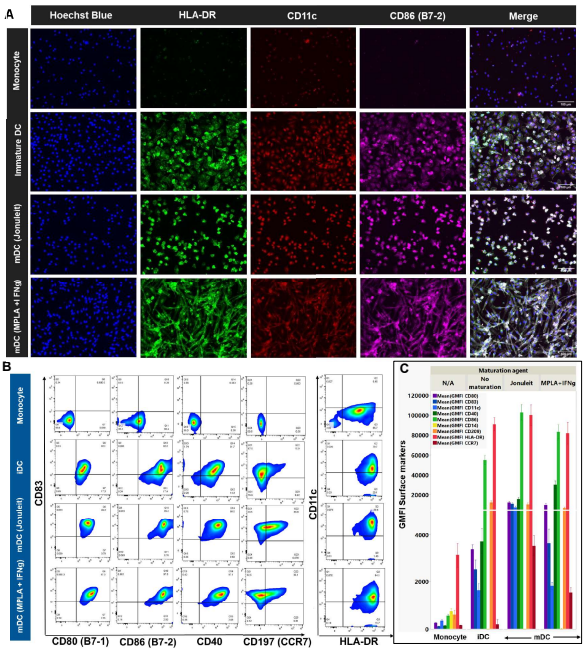

Fig. 5. Phenotypic characterization of DCs cultured in CTS AIM-V. (A) Representative immunofluorescent images showing HLA-DR (green), CD11c (red), CD86 (magenta), nuclei (blue) in monocytes, iDCs, mDCs (Jonuleit) and mDCs (MPLA + IFNg). Scale bar in merged images corresponds to 100 µm and applies to all images. (B) Representative flow cytometry dot plots showing various maturation markers on live monocytes, iDC, and mDC. (C) Bar graphs showing geometric mean fluorescence intensity of various surface markers on monocytes and DCs from 10 different donors. DC maturation markers (CD83, CD11c, HLA-DR); co-stimulatory molecules necessary for T-cell activation (CD80, CD86, CD40); adhesion molecules for T-cell engagement (CD209/DC-SIGN) and chemokine receptor CCR7 are significantly elevated while CD14 (monocyte marker) is downregulated in DCs cultured in CTS AIM-V as compared to monocytes. CCR7, CD83 and co-stimulatory molecule expression were elevated in mDCs as compared to iDCs.

Mixed leukocyte reaction (MLR) assay

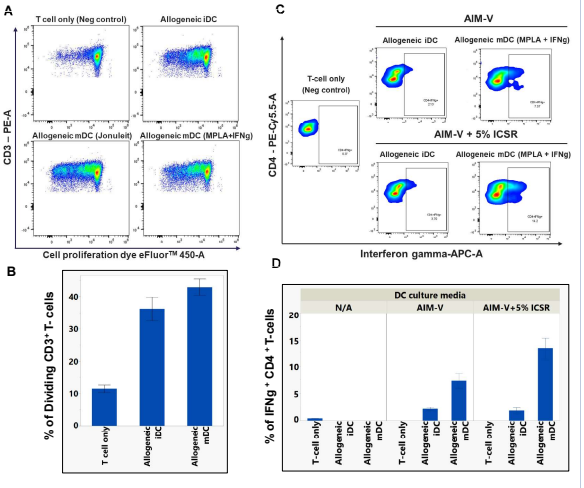

Fig. 6. DCs cultured in CTS AIM-V increase allogeneic T-cell proliferation and Th1 polarization. (A) Representative flow cytometry dot plots showing increased T cell proliferation after co-culture with DCs from HLA-mismatched donors. (B) Bar graph showing percentage of divided T–cells in allogeneic DC-T cell co-cultures. T-cells only with no DCs serve as negative controls. N=10 donors. (C) Representative flow cytometry dot plots showing increased interferon gamma staining in T helper cells in allogeneic mDC (MPLA + IFNg) – T- cell co-cultures. Supplementation of AIM-V with 5% ICSR increase interferon gamma positive T helper cells. (D) Bar graph showing percentage of interferon gamma positive T helper cells in allogeneic DC-T cell co-cultures. N=3-6 donors. Data represented as Mean ± Standard error of mean.

Cytokine secretion

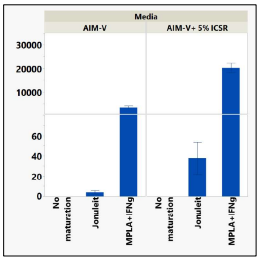

Fig. 7. Estimation of IL-12p70 levels in DC culture supernatants by ELISA. 5x10[5] monocytes were seeded in 1 mL of media / well on Day 0. IL-12p70 levels were obtained from iDC & mDC cell culture supernatants on Day 7. DCs matured by MPLA + IFNg significantly increase IL-12 secretion compared to traditional Jonuleit's cocktail. Supplementation of AIM-V with 5% ICSR significantly increased IL-12p70 levels as compared to AIM-V only. iDCs express very low to undetectable levels of IL-12p70. N=10 donors for Aim-V, N=4 for AIM-V+5% ICSR.

Autologous DC-T-cell co-culture antigen response assay

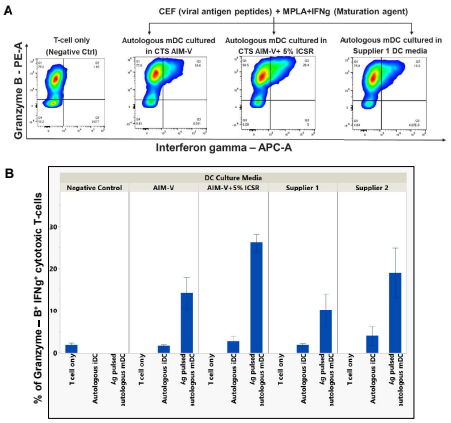

Fig. 8. Viral antigen pulsed mature DCs induce autologous CD8+ T-cell activation. A) Representative flow cytometry dot plots showing cytotoxic T-cell activation by viral antigen peptide mix (CEF) pulsed DCs matured with MPLA + IFNg as compared to T-cell only negative control. Activation was evidenced by significantly increased Granzyme B and interferon gamma double positive CD8+ T-cells. mDCs cultured in CTS AIM-V show comparable activation as compared to other supplier media. Supplementation of AIM-V with 5% ICSR further enhance CD8+ T-cell activation. B) Bar graph showing data pooled from 5-6 donors. Data represented as Mean ± Standard error of mean.

Cryopreserved DCs are viable and functional

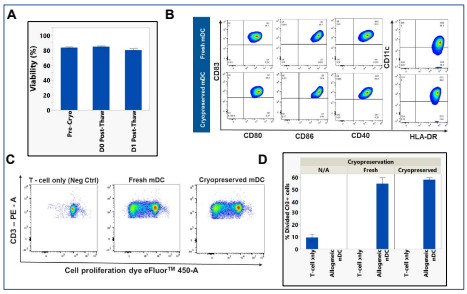

Fig. 9. Cryopreserved mDCs cultured in CTS AIM-V are viable, retain phenotypic markers, and are functional in inducing allogeneic T-cell proliferation. (A) Bar graph showing viability of mDCs (Jonuleit) before freezing with CTS PSC cryomedia, immediately after thawing, and after overnight culture in CTS AIM-V + IL-4 & GM-CSF. N=2 donors. (B) Representative flow cytometry dot plots showing phenotypic marker expression in freshly cultured and cryopreserved mDCs. (C) Representative flow cytometry dot plots and (D) Bar graph showing cryopreserved mDCs are functional in inducing allogeneic T-cell proliferation. N=2 donors.

CONCLUSIONS

CTS AIM-V is a suitable serum free medium for culture of DCs.
DCs cultured in CTS AIM-V show:

- Comparable or better yield than other suppliers
- Express desired markers of antigen presenting cells
- Enhanced functionality of mature DCs after CTS ICSR supplementation
- Robust T-cell proliferation and Th1 polarization in MLR assays
- Increased cytotoxic T-cell activation in antigen response assays
- Unaltered phenotype and function after cryopreservation
- Increased IL-12 production and T-cell activation with alternate DC maturation cocktails

REFERENCES

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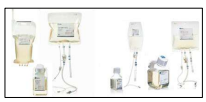
3. ten Brinke, A., van Schijndel, G., Visser, R. et al. Monophosphoryl lipid A plus IFNγ maturation of dendritic cells induces antigen-specific CD8+ cytotoxic T cells with high cytolytic potential. Cancer Immunol Immunother 59, 1185–1195 (2010). https://doi.org/10.1007/s00262-010-0843-z.

MARKETING INFORMATION



Product	Format	Catalog #
CTS™ AIM-V™ Medium	1 L bottle	A3639801
	2 L bag	A4072701
	10 L bag	A3830802
CTS™ Immune Cell Serum Replacement (ICSR)	50 mL bottle	A2596101
	250 mL bag	A4782901
	500 mL bottle	A2596102
	1 L bag	A4782902

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