Generation of Monocyte Derived Dendritic Cells Using Xeno-Free Clinical Grade Human Platelet Lysate

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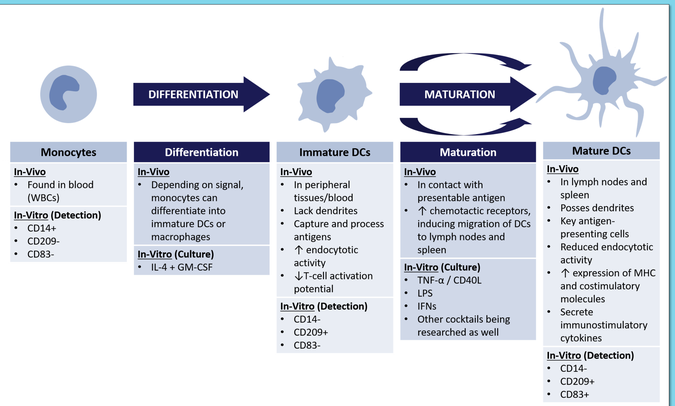
Introduction

Current Method of Mo-DC Generation: Dendritic cells (DCs), an important antigen-presenting cell type, have been widely used for therapeutic vaccine development in cancer immunotherapy. Among all of the approaches to obtain DCs, *ex vivo* generation from peripheral blood isolated monocytes has been the most favorable method. Briefly, isolated CD14+ monocytes are first differentiated into CD14-/CD209+ immature DCs, followed by a maturation process rendering the cells positive for CD83, CD86, HLA-DR, etc. The medium used in both phases of culture requires a serum supplement.



Limitations with Current Choices of Serum: Traditionally, clinical manufacturing of Mo-DCs has employed either fetal bovine serum (FBS), which poses potential risks for viral and prion transmission as well as adverse immunological reactions, or human AB serum (hABS), which is collected from a small number of donors and has considerable lot-to-lot inconsistency. These variations can significantly impact cell growth, morphology, and functionality, which is of concern with DCs because their phenotypic range is already diverse.

PLUS™ Human Platelet Lysate: To address these issues, Compass Biomedical has developed an alternative serum supplement for cell culture: PLUS™ human platelet lysate. PLUS™ is manufactured using a GMP production process with large lots of platelet units obtained from AABB-accredited blood banks, making it a more reliable alternative to hABS and a xeno-free alternative to FBS. The substantial availability of GMP-grade PLUS™, along with a Drug Master File (DMF) on record with the FDA, makes the production of clinical grade Mo-DCs for immunotherapy trials using PLUS™ a viable option.

Objective: This study aimed to demonstrate the validity of using PLUS™ to replace FBS and hABS in culture medium for differentiation and maturation of DCs from peripheral blood isolated monocytes.

Methods

Serum Product Origin: PLUS™ human platelet lysate was supplied in house by Compass Biomedical. Fetal bovine serum (FBS) was purchased from Corning and human Type AB serum (hABS) was purchased from MP Biomedicals.

Platelet Lysate Manufacturing: Expired platelet units were acquired from FDA-registered AABB accredited blood banks and lysed using a freeze-thaw process. Cell debris and clotting factors were removed. Each lot was produced by pooling >100 donors.

Monocytes Isolation: Peripheral blood mononuclear cells (PBMCs) were isolated from 3 healthy donors and selected for CD14+ monocytes using CD14 microbeads (Miltenyi Biotec).

Cell Culture: Freshly isolated monocytes were seeded at 10^6 cells/mL in RPMI-1640 medium supplemented with different concentrations of PLUS™, FBS or hABS, and differentiated to immature DCs by treating with IL-4 and GM-CSF for 4 days. Mature DCs were obtained via either TNF-α, IFN-ϒ or lipopolysaccharides (LPS) treatment for 2 additional days.

Flow Cytometry: Viable cell counting and immunophenotyping of CD14, CD209, CD83, CD86 and HLA-DR expressions were performed using a C6 Flow Cytometer (BD Accuri) with appropriate isotype controls. All antibodies were purchased from Miltenyi Biotec.

Mixed Lymphocyte Reaction (MLR) Assay: Carboxyfluorescein succinimidyl ester (CFSE, Invitrogen)-labeled human monocyte-depleted PBMCs were cultured at $4x10^5$ cells/well in a 24-well plate in 10% FBS-RPMI, with or without the addition of PLUS™, hABS and FBS-cultured mature DCs at a 1:10 ratio. T cell proliferation was determined after 6 days co-culture by flow cytometry analysis of CFSE fluorescence intensity.

Contact

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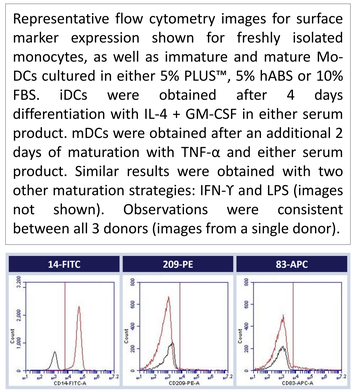
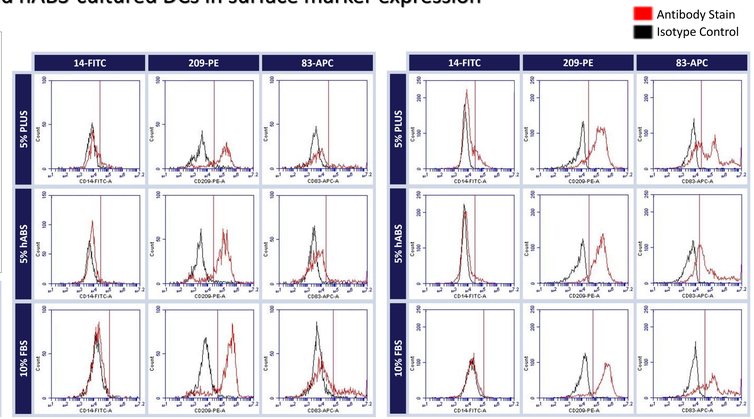
Results

PLUS™-cultured DCs emulate FBS and hABS-cultured DCs in surface marker expression

Representative flow cytometry images for surface marker expression shown for freshly isolated monocytes, as well as immature and mature Mo-DCs cultured in either 5% PLUS™, 5% hABS or 10% FBS. iDCs were obtained after 4 days differentiation with IL-4 + GM-CSF in either serum product. mDCs were obtained after an additional 2 days of maturation with TNF-α and either serum product. Similar results were obtained with two other maturation strategies: IFN-Y and LPS (images not shown). Observations were consistent between all 3 donors (images from a single donor).



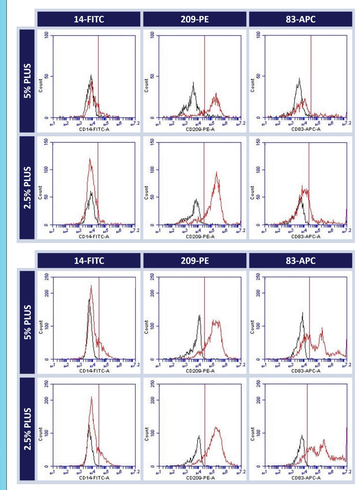
Freshly isolated monocytes were CD14+, CD209- and CD83-.



Immature DCs expressed negatively for CD14 and positively for CD209.

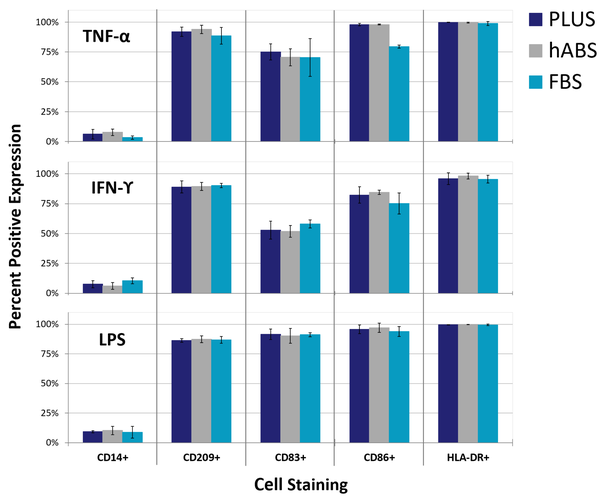
Mature DCs expressed negatively for CD14 and positively for both CD209 and CD83.

Consistent DC phenotype observed between PLUS™ concentrations



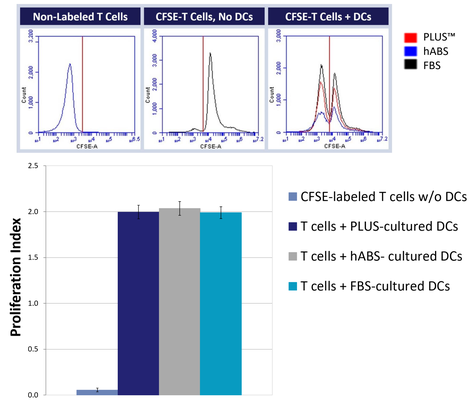
No differences in CD14, CD209 and CD83 expression were observed between 2.5% and 5% concentrations of PLUS™ for both differentiation and maturation of Mo-DCs. *Top Figure:* iDCs obtained after 4 days of differentiation in 2.5% and 5% PLUS™ with IL-4 and GM-CSF. *Bottom Figure:* mDCs obtained after an additional 2 days of maturation in 2.5% and 5% PLUS™ with TNF-α.

PLUS™ is compatible with multiple DC maturation pathways



Comparison of CD14, CD209, CD83, CD86 and HLA-DR expression results on mDCs cultured using either 5% PLUS™, 5% hABS or 10% FBS supplemented medium. mDCs were obtained by either of the three maturation pathways: TNF-α *(top)*, INF-γ *(middle)*; LPS *(bottom)*. N=3 donors for each test condition.

PLUS™-cultured DCs stimulate T cell proliferation



In a 6 day co-culture study, PLUS™-cultured Mo-DCs stimulated allogeneic T cell proliferation as efficiently as FBS and hABS-cultured Mo-DCs. *Top Figure:* T cell proliferation was traced by intracellular CFSE labeling, with labeled T cells in the absence of DCs serving as the negative control. *Bottom Figure:* The Proliferation Index was calculated as log[FInd/MFIall]/log[2], where MFIall = medium fluorescence intensity of all viable T cells and FInd = peak fluorescence intensity of the viable non-divided cells. No statistically significant difference in proliferation index was observed among any of the serum conditions. N=3 donors for each DC test condition.

Abbreviation List

DC: Dendritic Cell
Mo-DC: Monocyte-Derived Dendritic Cell
iDC: Immature Dendritic Cell
mDC: Mature Dendritic Cell

FBS: Fetal Bovine Serum
hABS: Human Type AB Serum

IL-4: Interleukin-4
GM-CSF: Granulocyte-Macrophage Colony Stimulating Factor
TNF-α: Tumor Necrosis Factor Alpha
IFN-γ: Interferon Gamma
LPS: Lipopolysaccharide
HLA-DR: Human Leukocyte Antigen – Antigen D Related
CFSE: Carboxyfluorescein Succinimidyl Ester

Discussion

- PLUS™ human platelet lysate can successfully replace FBS or hABS for large-scale *ex vivo* production of clinical grade Mo-DCs for immunotherapy trials.
- PLUS™ human platelet lysate produced mature DCs that were functionally equivalent to those cultured in FBS and hABS; being CD14-, CD209+, CD83+, CD86+ and HLA-DR+. Results were independent of maturation pathway chosen (TNF-α, IFN-γ or LPS).
- PLUS™-cultured mDCs had the same ability to stimulate T cell proliferation in vitro as FBS and hABS-cultured cells.
- **Future Work:** IL-12 p70 secretion, a commonly used *in vitro* indicator for functionally mature DCs, will be evaluated for PLUS™-cultured DCs.