Lipid Mediated Th1 Skewing of Human Dendritic Cells Cultured in a Chemically-Defined, Animal Component-Free Medium

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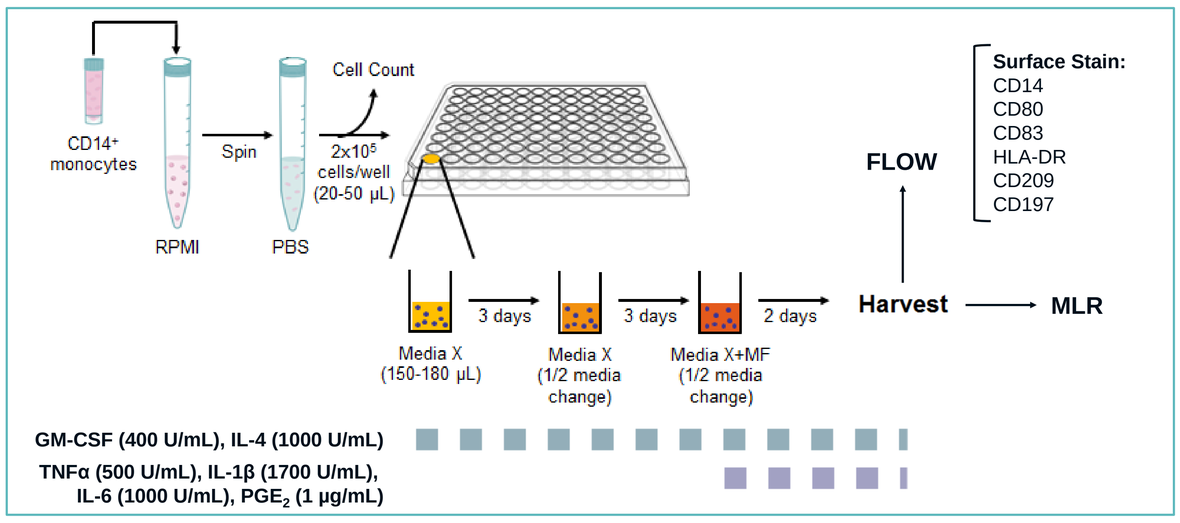
ABSTRACT

Monocyte-derived dendritic cells (Mo-DCs) have garnered much attention over the past 15 years in the efforts to develop effective strategies for therapeutic immunity against cancer. This is attributed to their central role in mediating the adaptive immune response and their ability to be manipulated *in vitro* at therapeutically relevant quantities. In this study, we outline a chemically-defined (CD), animal component-free (ACF) medium for the differentiation, maturation, and maintenance of Mo-DCs and demonstrate a Th1 polarizing effect upon addition of several chemically-defined lipids (CDL). Negatively selected CD14$^+$ monocytes were cultured for 6 days in the presence of GM-CSF and IL-4 to induce differentiation and subsequently cultured for 2 additional days in TNF-α, IL-1β, IL-6, and PGE2 to induce maturation for a combined culture duration of 8 days. Surface marker expression analysis confirmed the presence of mature Mo-DCs observed by down-regulation of CD14 and upregulation of CD80, CD83, HLA-DR, CD209, and CD197 expression. Minor changes in CD14 expression was observed upon CDL addition with variability across different donors. Mo-DCs generated with and without CDL were then co-cultured with naïve allogeneic CD4$^+$ T cells for 6 days to assay for functionality. CDL generated Mo-DCs demonstrated skewing toward Th1 observed by a dose dependent increase in IFN-γ expression. T cell stimulatory capacity was confirmed by loss of CFSE which was similar throughout all conditions. These results exhibit a new CD, ACF cultivation method able to favorably support immunogenic applications of Mo-DC and highlights a potential role for lipids in programming DC fate.

METHODS



RESULTS

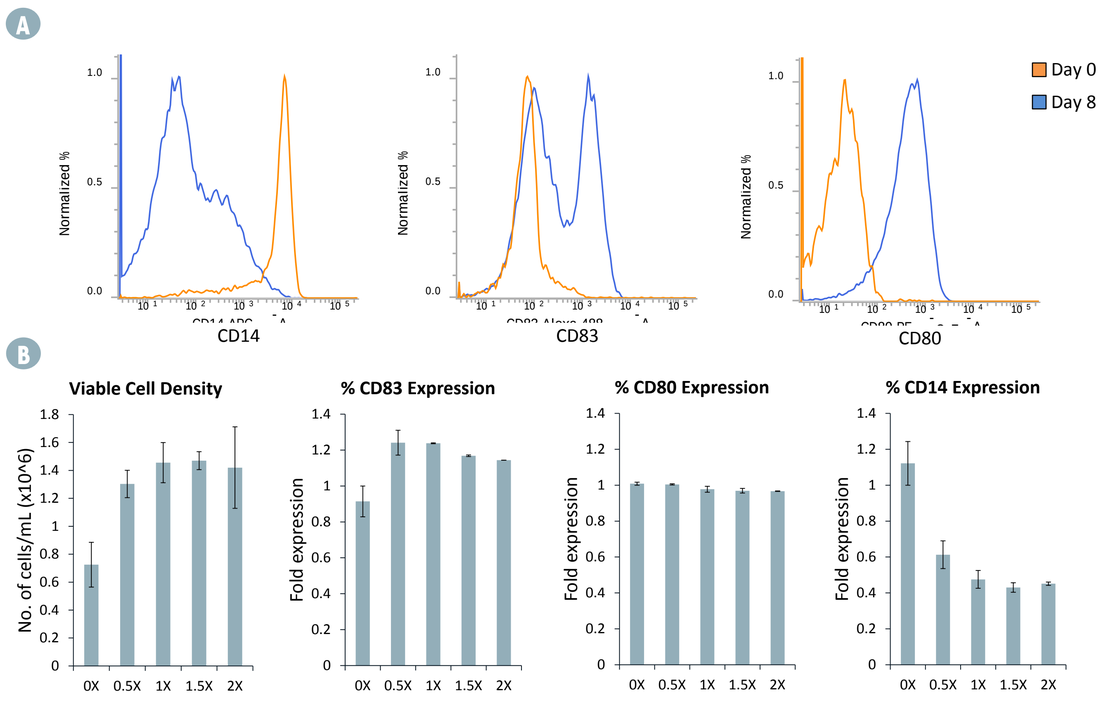


Figure 1. CD, ACF media formulation supports differentiation, maturation, and maintenance of Mo-DCs. Negatively selected CD14$^+$ monocytes were cultured for 6 days in the presence of GM-CSF and IL-4 to induce differentiation and subsequently cultured for 2 more days in TNF-α, IL-1β, IL-6, and PGE_2 to induce maturation for a combined culture duration of 8 days in each respective media. The resulting cells were harvested and analyzed by flow cytometry for viable cell density (propidium iodide exclusion and count beads) and surface marker expression (CD14, CD83, CD80). **(A)** Comparison of marker expression profile between Day 0 and 8 confirmed the downregulation of the monocyte marker CD14 and upregulation of dendritic cell markers CD83 and CD80 when cultured in the CD, ACF medium supplemented with 1X CDL. **(B)** Addition of CDL improved Mo-DC differentiation and maintenance demonstrated by increased viable cell density and reduced CD14 expression but no further improvement was observed with increasing CDL concentration (n=3). Fold expression represented as values normalized to CDL 0X.

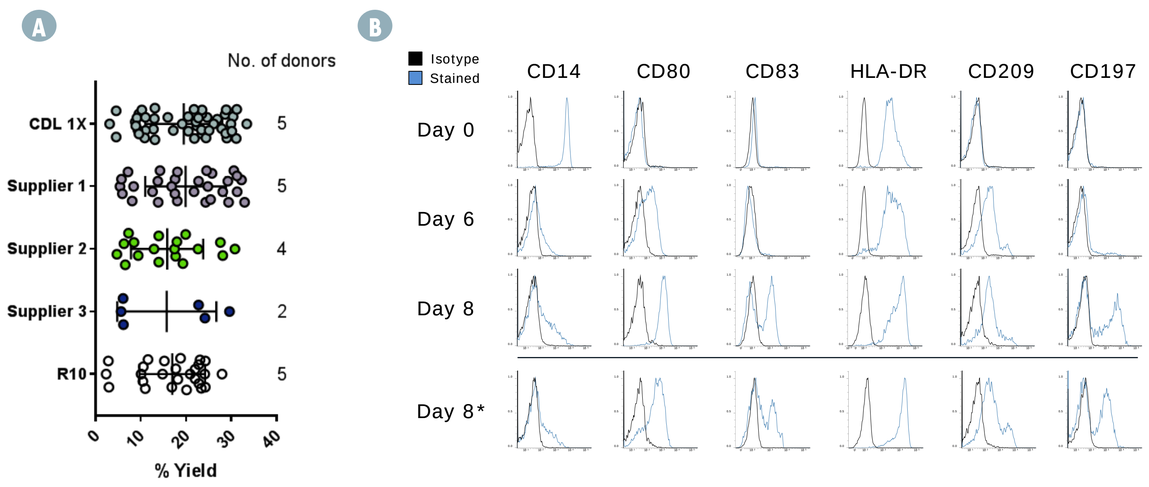


Figure 2. CD, ACF media formulation demonstrates comparable Mo-DC yield to other supplier media. Negatively selected CD14$^+$ monocytes were cultured for 6 days in the presence of GM-CSF and IL-4 to induce differentiation and subsequently cultured for 2 more days in TNF-α, IL-1β, IL-6, and PGE_2 to induce maturation for a combined culture duration of 8 days in each respective media. The resulting cells were harvested and analyzed by flow cytometry for viable cell density (propidium iodide exclusion and count beads) and surface marker expression. **(A)** Comparable Mo-DC yield utilizing up to 5 donors was observed across various media conditions tested. R10: RPMI + 10% FBS. **(B)** Surface marker expression profile at day 0 (monocyte), day 6 (iDC), and day 8 (mDC). Comparable expression profile was observed with other leading suppliers' chemically-defined, animal component-free media (day 8*).

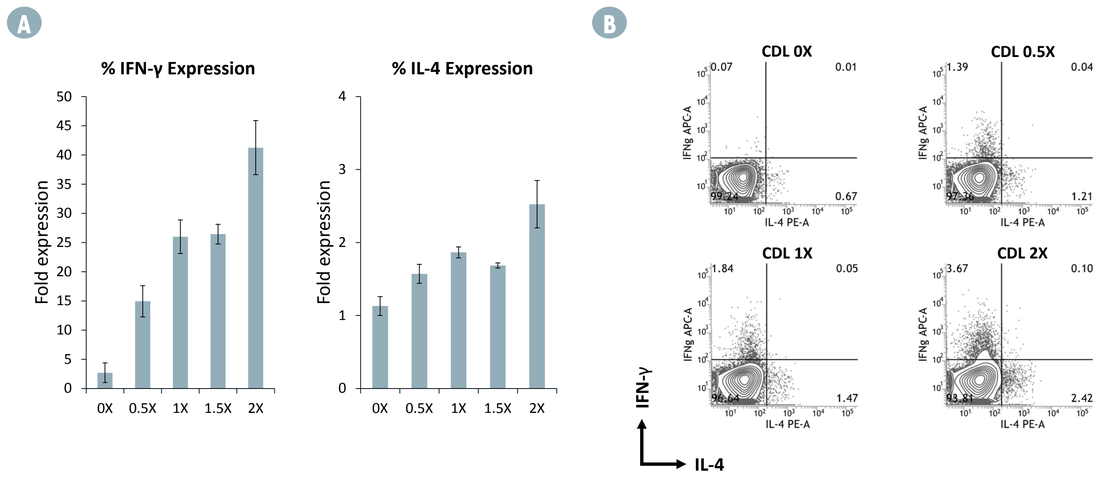


Figure 3. CDL generated Mo-DCs demonstrate Th1 skewing of allogeneic CD4$^+$ T cells. Day 8 Mo-DC cultured in increasing CDL concentration were harvested and co-cultured with allogeneic CD4$^+$ T cells at a 1:20 (DC to T) ratio and cultured in a commercially available T cell medium supplemented with IL-2 for 6 days prior to analysis. **(A-B)** Flow cytometry analysis demonstrated a notable dose dependent increase in IFN-γ as well as a minor increase in IL-4 expression (n=3). Fold expression represented as values normalized to CDL 0X.

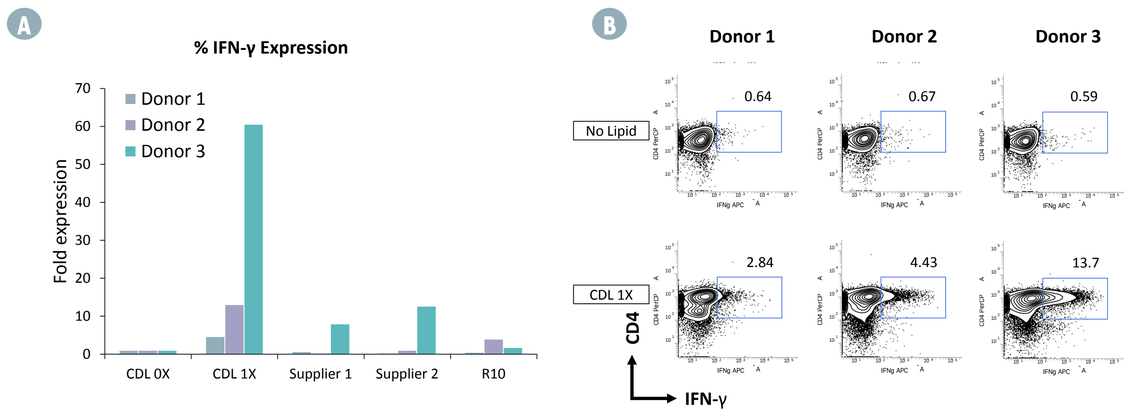


Figure 4. CDL mediated Th1 skewing is observed across varying donors. Day 8 Mo-DC cultured in each respective media were harvested and co-cultured with allogeneic CD4$^+$ T cells at a 1:10 (DC to T) ratio and cultured in a commercially available T cell medium supplemented with IL-2 for 6 days prior to analysis. **(A-B)** Flow cytometry analysis demonstrated an increase in IFN-γ expression across three different donors compared to R10 (serum control) and two other commonly utilized suppliers. A moderate level of donor variability was observed in IFN-γ expression but was consistently highest in CDL derived Mo-DC. Fold expression represented as values normalized to CDL 0X. R10: RPMI + 10% FBS.

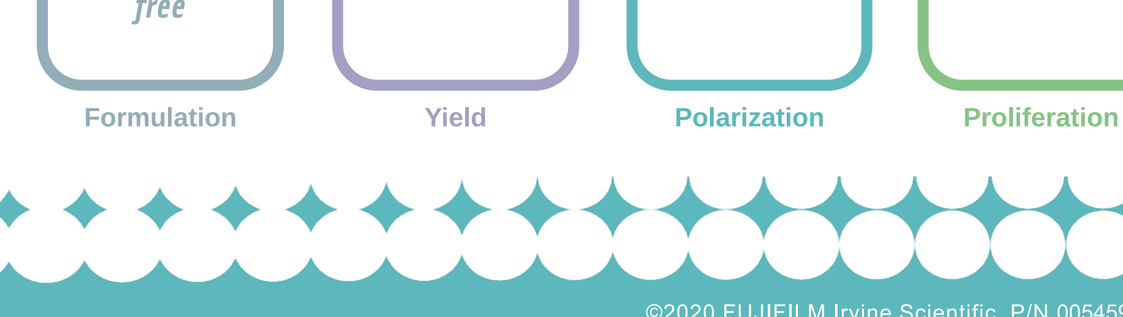


Figure 5. CDL derived Mo-DCs support alloreactive expansion of CD4+ T cells. Day 8 Mo-DC cultured in each respective media were harvested and co-cultured with allogeneic CD4$^+$ T cells at a 1:20 (DC to T) ratio and cultured in a commercially available T cell medium supplemented with IL-2 for 6 days prior to analysis. **(A-B)** T cell stimulatory capacity was confirmed by loss of CFSE which was similar throughout all conditions tested (n=3). CD4$^+$ T cells cultured without Mo-DC were cultured in IL-2 alone or supplemented with anti-CD28 to serve as negative and positive controls, respectively. R10: RPMI + 10% FBS.

SUMMARY