Modeling the cDC1 ex vivo and in vitro: Development and characterization of a conventional dendritic cell culture system for industry

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ABSTRACT # 4230

INTRODUCTION

- Conventional dendritic cells (cDC) are innate immune cells specialized in antigen sampling.
- Subsequent cross-presentation to immune cells makes these cells critical for an effective anti-tumor immune response.

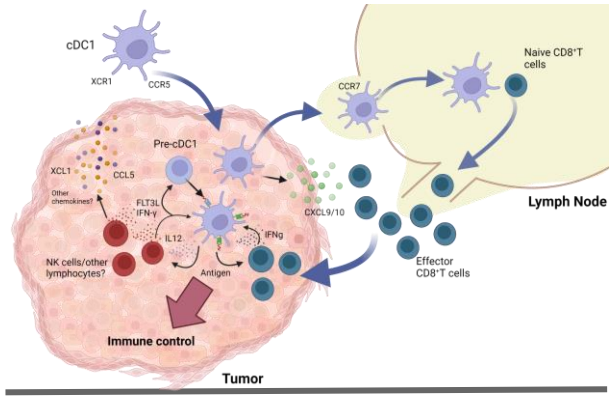


Figure 1: Adapted from Orchestration of Cancer Immune Control by cDC1. (Böttcher and Reis e Sousa, 2018)

- cDC type I (cDC1) are a relatively rare population and constitute about 0.03% of cells in the blood, making these cells a challenge to model as part of in vitro screening studies.
- Existing methods to generate acceptable numbers of cDC1s require a feeder cell line, which is not accessible to many laboratories.

METHODS

In vitro differentiation of CD34⁺ Cord Blood cells into cDC1

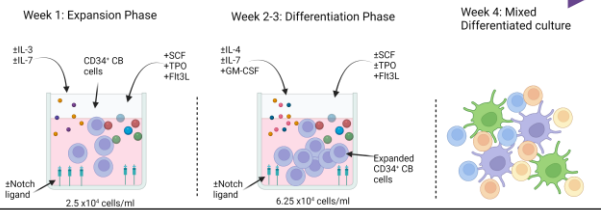


Figure 2: Conventional dendritic cells are derived from CD34⁺ Cord blood cells. Dependent on assay conditions per the experiment, media base is either alpha-MEM+Glutamax containing 10%FBS, 1mM Sodium Pyruvate, and 50uM β-mercaptoethanol (referred to as αMEM) or Stemspan +10%FBS for expansion phase and then RPMI-1640 +10%FBS differentiation phase (referred to as SS). Wells were either coated with 1µg/mL Dll1 human notch ligand, added solubly at 10µg/mL in culture media or not. Cells were cultured at $2.5x10^4$ cells/mL (expansion phase) or $6.25x10^4$ cells/mL (differentiation phase) in media containing specified growth factors. Half media changes were performed 5 days after cells were placed in differentiation media at a 2x cytokine concentration.

RESULTS

Medium base, cytokine cocktail and Notch signaling affect expansion and differentiation of CD34⁺ cells into cDC1

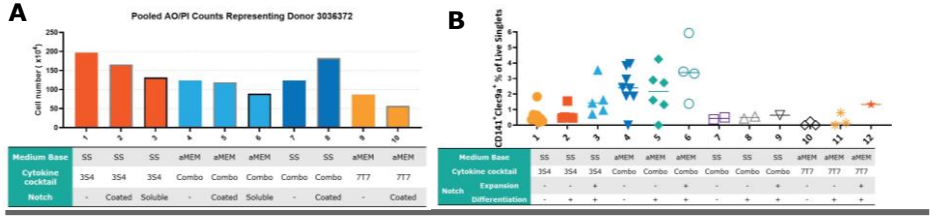
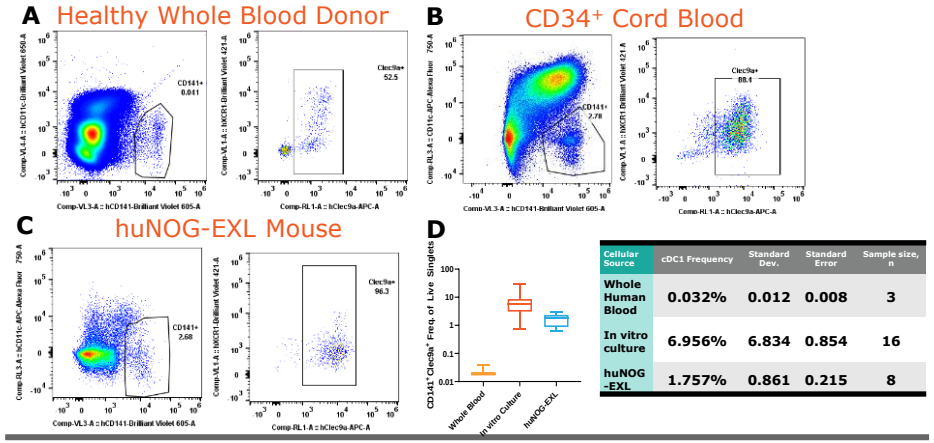


Figure 3: Medium base, cytokine cocktail composition, and provision of Notch ligand all affect the ability of CD34⁺ cells to differentiate into cDC1 cells. (A) CD34⁺ cord blood cells expand best when cultured using Stemspan medium and the 3S4 cytokine cocktail without the provision of Dll1 Notch ligand. High numbers are also observed when CD34⁺ cord blood cells are cultured with Stemspan medium and the Combo cytokine cocktail. Cell number represents the total cell count and does not include phenotypic analysis. (B) Each symbol represents a unique donor cultured under these conditions. While there is donor to donor variability in proportions of CD141⁺ Clec9a⁺ cells, highest proportions are observed with αMEM medium base, in the absence or presence of plate coated Dll1 notch ligand in either the differentiation phase or during both phases. This systematic approach of optimizing culture conditions to best achieve the highest proportion of CD141⁺ Clec9a⁺ cells suggests that the αMEM medium base, supplemented with Combo cytokine cocktail is the optimal condition although it may not yield the most cells.

In vitro differentiated cDC1 cells and cDC1 cells isolated from humanized mice are phenotypically similar to those found in whole blood



Cellular Source	cDC1 Frequency	Standard Dev.	Standard Error	Sample size, n
Whole Human Blood	0.032%	0.012	0.008	3
In vitro culture	6.956%	6.834	0.854	16
huNOG-EXL	1.757%	0.861	0.215	8

Figure 4: Expression of prototypical cDC1 markers (CD141 and Clec9a) are evaluated on cells in whole blood from a healthy donor (A), from an in vitro differentiation culture system (B), in spleen homogenate from a huNOG-EXL mouse (C). Proportions of CD141⁺ Clec9a⁺ cells are higher in the in vitro differentiation system compared to those present in whole blood or isolated from huNOG-EXL mice (D). Each symbol represents a single donor. Using flow cytometry, we identify cDC1s based on CD11c⁻ or CD11c^{lo}, CD141⁺, and Clec9a⁺ gating. These data provide support that the in vitro culture conditions defined (αMEM + Combo cytokine cocktail with Dll1 notch ligand) generate phenotypically similar cDC1 cells in much higher proportions than any other source of cDC1 cells.

RESULTS

In vitro differentiated cDC1 cells and cDC1 cells isolated from humanized mice are functionally responsive

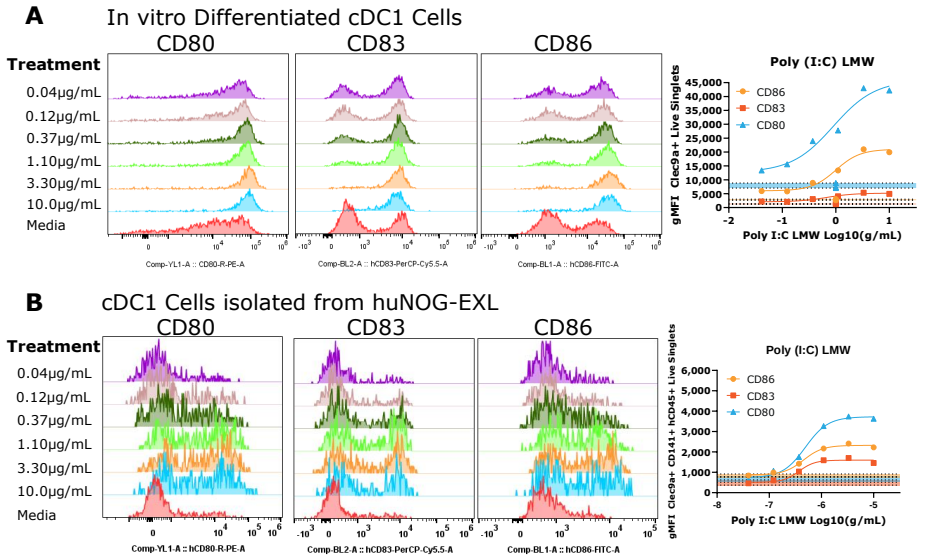


Figure 5: In vitro differentiated cDC1 cells are functionally active as defined by upregulation of CD80, CD83, and CD86 following stimulation with poly (I:C) treatment. Using flow cytometry, upregulation in activation markers were observed in cells that are CD11c⁻ or CD11c^{lo}, CD141⁺, and Clec9a⁺ gating. These data suggest that in vitro differentiated cDC1 cells are functionally active.

CONCLUSION

- **Model developed using CD34⁺ cord blood cells to reproducibly generated phenotypic cDC1s in the absence of a feeder cell line**
- **Identified an alternative model utilizing cells from humanized mice as a source of cDC1s with the potential to be activated by Poly (I:C)**
- **In vitro culture results in cDC1 cells responsive to stimulus Poly (I:C), leading to upregulation of CD80, CD83, and CD86**

References: Böttcher and Reis e Sousa, Trends in Cancer. 4:784-792 (2018)

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