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An 8-color DuraClone IM panel for detection of Human blood dendritic cells by flow cytometry

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INTRODUCTION

Dendritic cells (DCs) are antigen presenting cells capable of priming a T cell response. They form a heterogeneous group of cells based on phenotype, location and function. In human blood, DCs represent less than 1% of white blood cells, and can be separated into 2 main cell subsets, namely the myeloid DCs (MDCs) and the plasmacytoid DCs (pDCs). Among the mDCs, 3 distinct cell subsets are identified: $CD1c^{+}$MDCs (MDC1), $CD141^{+}$mDCs (MDC2) and $CD16^{+}$mDCs. In blood, the frequency of DCs is affected in certain pathological conditions such as HIV, diabetes, asthma, chronic viral hepatitis, and graft-versus-host- disease. Thus, the detection and enumeration of different blood DC subsets is important to understand immune regulation in challenging conditions. Due to the lack of specific markers for DC definition, the combination of several markers is required to allow their identification. Based on current knowledge in human DC biology, we have evaluated the expression and association of several DC markers to design an optimized 8-color panel for flow cytometry which allows for the detection of all DCs subsets in whole blood samples or peripheral blood mononuclear cells (PBMCs). This panel (CD1c/HLA-DR/Lineage/CD11c/ CD16/Clec9A/CD123/CD45) provides an easy and robust assay to study the role of DCs in the human immune system. To improve assay reproducibility and ease the laboratory workflow and assay reproducibility a DuraClone version, namely IM-Dendritic cells, was generated and evaluated.

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1. DC subsets and detection by Flow Cytometry

DC subsets definition is based on the expression of surface markers. An initial gating is applied on high expression of HLA-DR and lack of Lineage (Lin) surface markers from other immune cells such as CD3 (T cells), CD14 (Monocytes), CD19 and CD20 (B cells) and CD56 (NK cells). Among those cells, the expression of CD11c allow a first separation between the 2 main DC lineages, i.e plasmacytoid DCs (pDCs, CD11c-) and Myeloid DCs (MDCs, $CD11c^+$). Among MDCs and PDCs other surface markers allow a further discrimination in different subsets (Table1)

Subsets	Function	Phenotype	% of $HLA\text{-}DR^+Lin^-$ cells (1)
MDC1	$CD4^+$ T cells activation	$CD11c^+CD1c^+$	18.6% +/- 7.6%
MDC2	$CD8^+$ T cells activation (cross presentation)	$CD11c^+CD141^+Clec9A^+XCR1^+Necl2^+$	2.7% +/- 1.4%
$CD16^+$ MDCs	Inflammatory DCs	$CD11c^+CD16^+$	49.6% +/- 8.5%
pDCs	Antiviral DCs	$CD11c^-BDCA2^+CD123^+$	18,3% +/- 9.7%

Table 1: DC subset phenotype

2. Identification of MDC2s using Clec9A (DNGR-1)

MDC2s, the least frequent MDC subpopulation in peripheral blood, constitutes a key subset due to its importance in immunity to pathogens/tumors, vaccines and tolerance to self. However, most functional characterizations of MDC2s refer to studies performed in mouse models. Until recently, the role of MDC2s in human biology remained hypothetical since there was no alignment of DC subsets between the two species. Several newer studies have shown that human MDC2s share several surface molecules, such as Clec9A, NECL2 or Xcr1, and functional capabilities with the mouse $CD8^+$ mDCs allowing for cross-species interpretation of MDC subset functions between mouse and human. Using a 6-color panel (Displayed below), we evaluated the use of Clec9A (DNGR-1) to identify MDC2 by flow cytometry in comparison to the currently used CD141 (BDCA-3). As shown in a representative sample (Figure 1A), CD141 and Clec9A are co-expressed on CD11c-HLADR-Lin-cells. The analysis of 9 independent blood samples, shows excellent correlation between the expression of these two markers (r^2=0,9248, Figure 2B). Moreover, the recruitment of MDC2s cells based on the expression of CD141 (BDCA-3) or Clec9A does not show significant differences (Figure 1C).

Excitation Laser	405 Excitation		488 Excitation					633 Excitation		
Dye	Pacific Blue	Krome Orange	FITC	PE	ECD	PC5.5	PC7	APC	APC-A700 (1)	APC-A750 (2)
Specificity	HLA-DR	CD45	CD141 (BDCA3)	Lineage-PE cocktail	-	-	CD11c	Clec9A	-	-

Table 2: 6 color panel to evaluate Clec9a and CD141 expression redundancy

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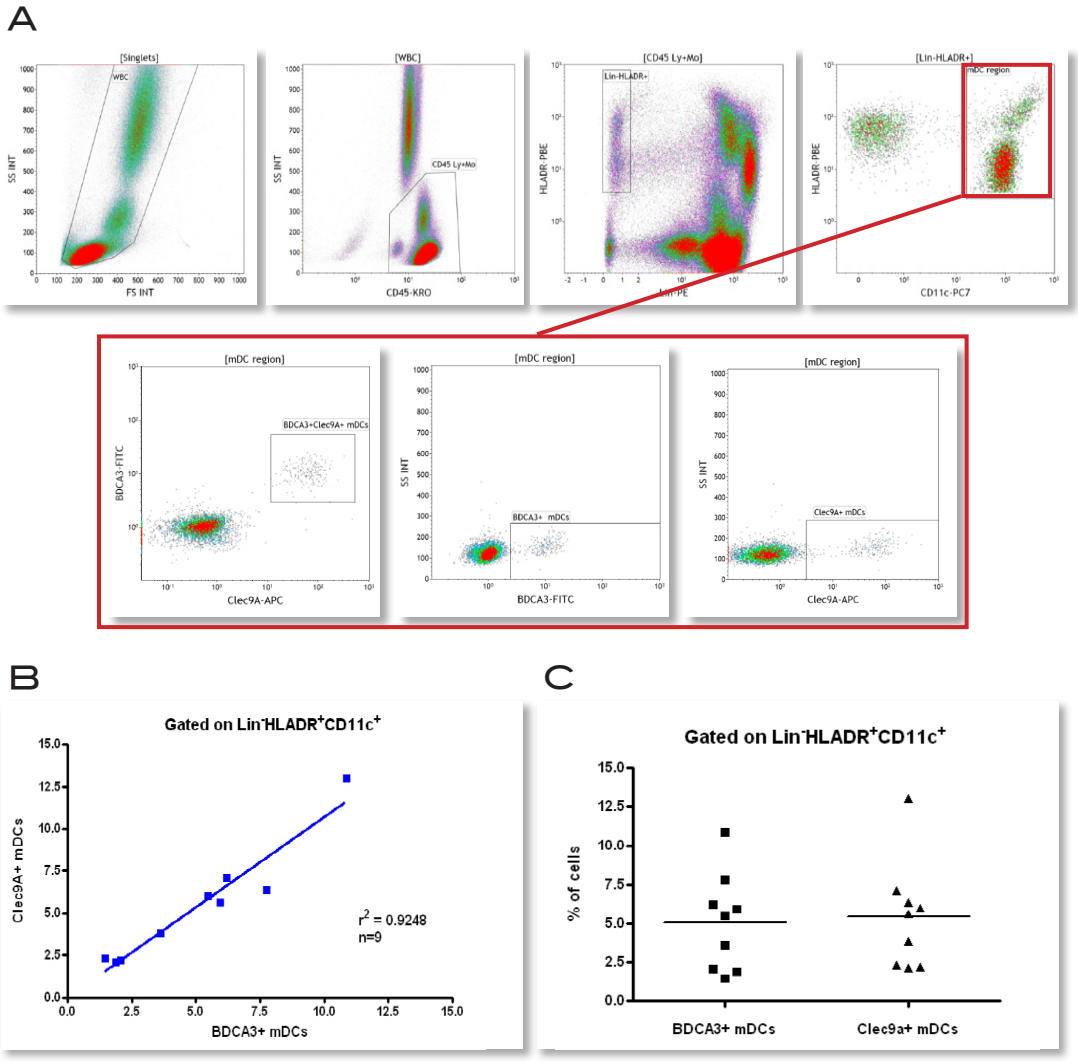

Figure 1 : Characterization of MDC2 phenotype.

a: representative staining and gating strategy used for analysis

b: Graph depicting the correlation between the recruitment of Clec9A+mDCs and BDCA3+ mDCs.

c: Recruitment of cells of interest.

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3. Eight-Color Panel for Identification of Dendritic cells

Based on the results of the previous experiment an 8 color panel for detection of blood circulating DC by flow cytometry was evaluated.

Excitation Laser	405 Excitation		488 Excitation					633 Excitation		
Dye	Pacific Blue**	Krome Orange	FITC	PE	ECD	PC5.5	PC7	APC	APC-A700 (1)	APC-A750 (2)
Specificity	**HLA-DR** (Immu-357)	**CD45** (J33)	**CD16** (3G8)	**Lineage-PE** (UCHT1, RMO52, J3-119, B9E9 (HRC20) and N901)	-	**CD1c** (L161)	**CD11c** (BU15)	**Clec9A** (8F9)	**CD123** (SSDCLY107D2)	-

A gate on Lymphocytes and Monocytes, indicated as CD45+ Ly/Mo, is identified in a dot plot for CD45 versus Side Scatter (I). These events are displayed in a HLA-DR versus Lineage dot plot where a gate selects Lineage negative/HLADR+ cells (II). Among these cells, a CD123 versus CD11c dot plot allows for identification of plasmacytoid dendritic cells (CD123+ cells) and myeloid dendritic cells (CD11c+ cells). The radar plot visualizes the 3 distinct subsets of MDCs (IV).

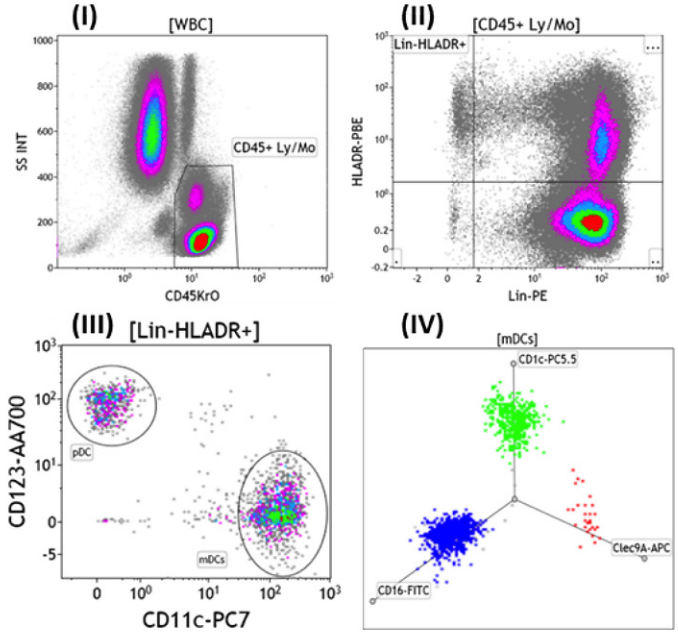

Figure 2 : Gating strategy for DC subsets identification. Cytometry staining was performed on whole blood samples using Versalyse according to IFU. Samples were acquired on a Gallios* Cytometer (3 lasers, 10 colors) and data were analyzed with Kaluza* software (Beckman Coulter).*

(1) APC-Alexa Fluor** 700 (2) APC-Alexa Fluor** 750

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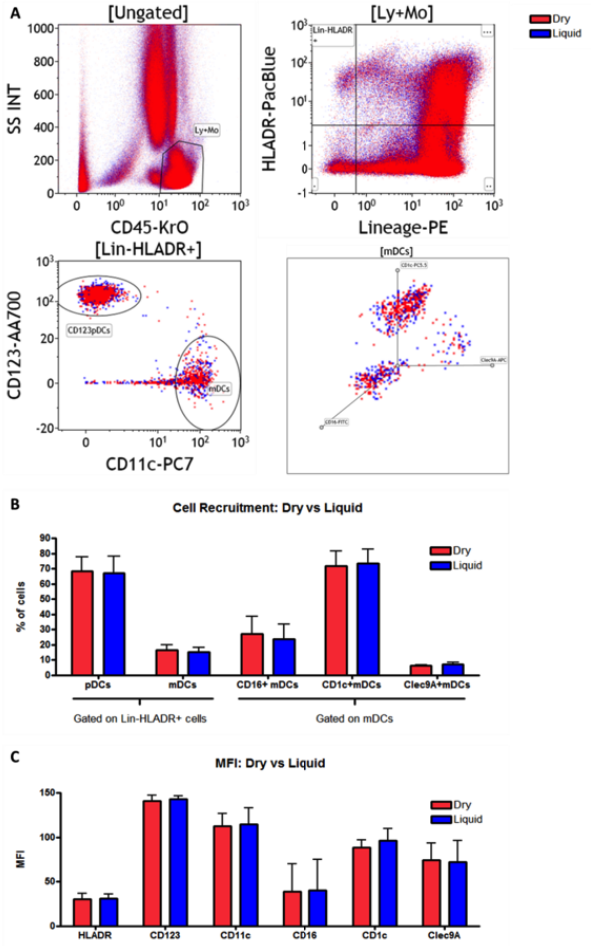
4. IM-Dendritic cells: Dry unitized panel for DC detection

Recently, Beckman Coulter has demonstrated with the DuraClone IM Phenotyping Basic Tube 25 tests RUO (*Part # B53309*) that the DuraClone format is a powerful tool for standardization of flow cytometry application by improving the workflow, stabilizing antibody conjugates and allowing long time storage of antibody conjugates at room temperature («DuraClone reagents are a robust solution for standardizing multicolor flow cytometry applications» *Nathalie Dupas[1], Snehita Sattiraju[2], Neha Girish[2], Emmanuel Gautherot[1], Sridhar Ramanathan[2], Tewfik Miloud[1]*). Here, the established DC panel has been dried down and it is evaluated in comparison with the liquid counterpart. As shown, in Figure 3A, whole blood samples stained with the DuraClone panel display the same staining than the liquid panel. Analysis of the cell recruitment (Figure 3B) and the mean fluorescence intensity (Figure 3C) of the DC specific markers confirms that the DuraClone format is comparable with the liquid marker panel.

Figure 3: IM-Dendritic cells : DuraClone compared with liquid counterpart on 3 independent donors and results are comparable



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CONCLUSION

The importance of analytical methods to identify dendritic cells subsets in whole blood samples to reflect the immunocompetence of individuals in various conditions is well described. Based on the latest knowledge of DC biology, we have designed an 8-color panel, namely the IM-Dendritic cell, for detection of human blood dendritic cells by flow cytometry. Usage of the unitized DuraClone format simplifies the workflow and minimizes variability in clinical research studies.

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