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

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Abstract / Introduction

Dendritic cells (DCs) are antigen presenting cells capable of presenting antigen and 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 pathological conditions and to guide specific patient treatments. 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 healthy donors and patient samples.

1. DC subsets and detection by Flow Cytometry

DC subsets definition is based on the expression of surface markers. An initial gating is made 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 allows 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). Cytometry staining were 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).

Subsets	Function	Phenotype	% in PBMCs
MDC1	CD4+ T cells activation	CD11c$^+$CD1c+	0,3% - 0,8%
MDC2	CD8+ T cells activation (cross presentation)	CD11c$^+$CD141$^+$Clec9A$^+$XCR1$^+$Necl2$^+$	0,02% - 0,06%
CD16 $^+$ MDCs	Inflammatory DCs	CD11c$^+$CD16$^+$	0,75% - 2%
pDCs	Antiviral DCs	CD11c$^-$BDCA2$^+$CD123+	0,3% - 0,8%

Table 1: DC subset phenotype

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

Even if MDC2s are the less represented MDCs in whole blood, they constitute a key subset due to their importance in immunity to pathogens/tumors, vaccines and tolerance to self. However, most functional characterizations of MDC2s are extrapolated from studies performed on mouse models. Until recently, the translation to human biology remained hypothetical since no alignment of DC subsets was done between the two species. Recently, several studies have shown that human MDC2s share several surface molecules, such as Clec9A, NECL2 or Xcr1, and functional capabilities with the mouse CD8$^+$ mDCs[a] allowing clear interferences to be drawn between mouse and human. Thus, we evaluated the use of Clec9A (DNGR-1) to identify CD141 MDCs by flow cytometry in comparison to the currently used CD141(BDCA-3). The results obtained indicates that Clec9A is an efficient marker for the identification of MDC2s.

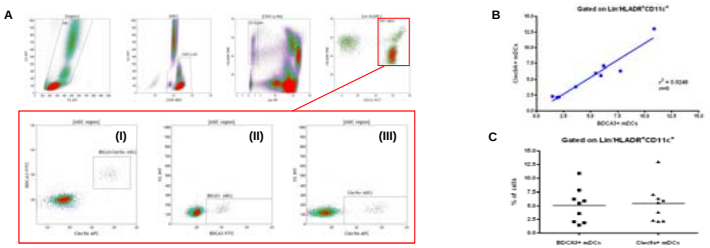


Figure 1: Characterization of MDC2 phenotype. In the same multicolor staining of 9 whole blood samples from healthy donors, the expression of Clec9A and CD141(BDCA-3) was evaluated. The correlation between the expression of both markers is very good (r^2=0,9248; B). Additionally, the recruitment of MDCs based on the expression of BDCA-3 (A.II) or Clec9A (A.III) is non significantly different (C).

3. Identification of pDCs

BDCA2 and CD123 are commonly used in combination to identify pDCs among the Lin-HLADR+CD11c- cells (Fig1A.I). In order to optimize the multi-color panel and avoid the usage of a fluorescent channel with a marker that will be redundant, we have evaluated the correlation between BDCA2 and CD123 expression on Lin-HLADR+CD11c- cells of 22 healthy donors. We show that one of the two marker is sufficient to identify pDCs. Because CD123 is also useful for the identification of basophils on Lin-HLADR- cells, we have chosen to use CD123 in further experiments for the identification of pDCs.

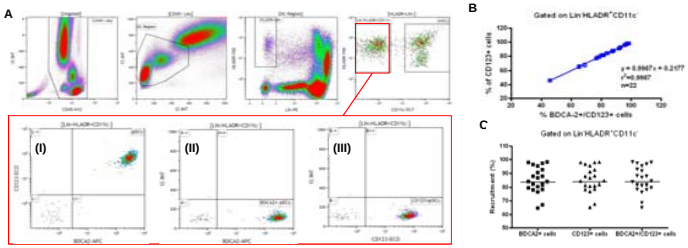
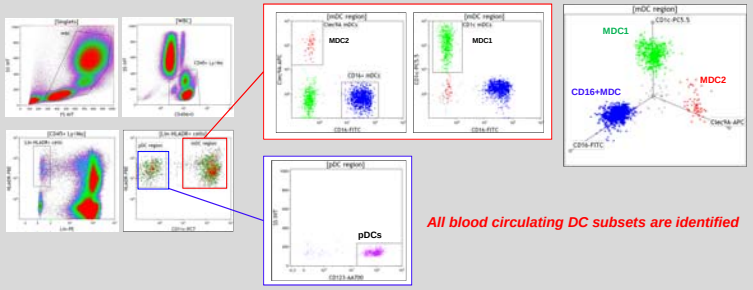


Figure 2: Characterization of pDCs. The correlation between the expression of the BDCA-2 and CD123 is very good (r^2=0,9987; B). Additionally, the recruitment of pDCs is identical whether BDCA2 and CD123 are used in association or individually (A and C) indicating that the expression of this two markers is redundant on pDCs.

4. 8-Color Panel for Identification of Dendritic cells

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

Excitation Laser	405 nm		488 nm					638 nm		
Dye	Pacific Blue*	Krome Orange*	FITC	PE	ECD	PC5.5	PC7	APC	APC-Alexa FLuor700**	APC-Alexa FLuor750**
Specificity (Clone)	HLA-DR (Immu-357)	CD45 (J.33)	CD16 (3G8)	Lineage-PE cocktail (UCHT-1, RMO52, J3-119, B9E9 and N901)		CD1c (L161)	CD11c (BU15)	Clec9A (8F9)	CD123 (55DC1Y107D2)	



All blood circulating DC subsets are identified

Figure 3: Gating strategy for DC subsets identification. The 8-color panel was used for the staining of whole blood samples from healthy donors.

5. External evaluation of the panel

Measuring or monitoring the frequency of the various blood circulating DCs subsets is potentially important for the establishment of a diagnostic or the evaluation of susceptibility to therapeutic treatments. As an example, the 8-Colors panel was evaluated in the Institute of Medical immunology (Charité, Berlin). The panel was applied to evaluate the level of blood circulating DCs of patients with Chronic Kidney Disease (CKD). As shown in Figure 4, all DC subsets are affected in the CKD patients. MDC1, MDC2 and pDCs are less represented in comparison to the control group while CD16$^+$ MDCs are increased in percentage. This observation shows that even for rare population differences could be assessed in whole blood samples without the requirement of cell enrichment prior to staining.

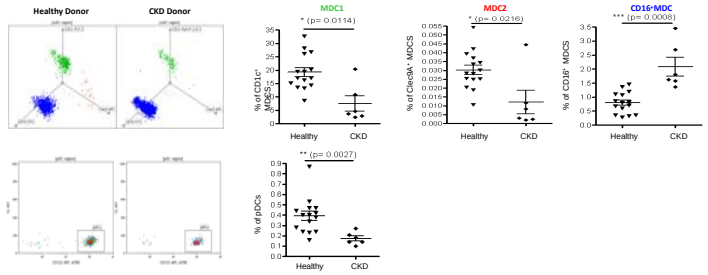


Figure 4: Identification of DC subsets in CKD patients. The 8-color panel was use for the staining of whole blood samples from CKD patients (n=6) and aged matched healthy donors (n=15). The percentage of the different DC subsets is expressed relatively to Lymphocytes/Monocytes population.

Conclusion

- All 4 blood circulating DC subsets can be identified
- CD123 is sufficient to identify pDCs when used in combination with CD11c and HLA-DR
- Clec9A can be used for the identification of MDC2s thus facilitating the translation between human and mouse models
- Application to CDK shows that detection of DC variation can be performed on whole blood samples

[a] Jose A. Villadangos and Ken Shortman. Found in translation : The human equivalent of mouse CD8$^+$dendritic cells. J. Exp. Med Vol. 207 No.6 1131-1134.