Immunophenotyping of Human Dendritic Cell Population

Multicolor flow cytometry to identify and characterize human dendritic cell populations in peripheral blood

Dendritic cells (DCs) are a class of innate immune cells that originate from bone marrow and are distributed in multiple lymphoid organs, tissues and blood. DCs play a crucial role in innate and adaptive immunity and are recognized as the antigen presenting cell (APC) that prime naïve T cells and elicit memory T cell responses to foreign antigen. Here, we aim to demonstrate use of a multicolor flow cytometry panel for analyzing DC populations in human blood using our 30-parameter BD FACSymphony™ A3 Cell Analyzer.

The multicolor flow cytometry panel described here was comprised of 20 fluorochrome-labeled antibodies including BD Horizon Brilliant™ Reagents. Using this multicolor panel, we documented the phenotype and prevalence of various DC subsets in peripheral blood mononuclear cells (PBMCs) from healthy human subjects. DCs can reportedly be classified as classical or conventional DC subset 1 (cDC1), classical DC subset 2 (cDC2), and non-classical DC.[1-3] The filter configuration of the BD FACSymphony A3 Cell Analyzer offers flexibility to assign fluorochromes across five lasers that facilitated distinction of not only the classical and non-classical DC subsets but also unique DC subsets such as Axl^+ $Siglec6^+$ DC (AS DC) and plasmacytoid dendritic cell (pDC).[2]

Protocol

Fresh PBMCs were isolated from the whole blood of healthy donors (n = 3) by Ficoll™ Paque Plus (Cytiva) gradient centrifugation. An antibody cocktail was prepared using the BD Horizon™ Brilliant Stain Buffer Plus mixed with specified test volumes per reagent (Table 1). PBMCs were stained with the antibody cocktail for 30 minutes at room temperature (RT) in the dark. Single color and fluorescence minus one (FMO) controls were processed in parallel with the full panel. After staining, cells were washed three times with BD FACS™ Stain Buffer and acquired using a BD FACSymphony A3 Cell Analyzer. Data were re-analyzed using FlowJo™ v10.7 Software.

Table 1. Instrument configuration and reagent selection

Cell surface markers and antibody clones used in the cocktail for the multicolor DC panel are shown in Table 1. The fluorochromes have been assigned based on a BD FACSymphony A3 Cell Analyzer configured with five lasers and 28 fluorescence detectors.

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Ultraviolet 355 nm	CD11c	BUV395	B-ly6	5 µL	563787
	CD172a/b (SIRPα/β1)	BUV496	SE5A5	2.5 µL	749939
	CD303	BUV563	V24-785	5 µL	748415
		BUV615			
		BUV661			
	CD86	BUV737	2331 (FUN-1)	5 µL	612784
		BUV805			
Violet 405 nm	CD327 (Siglec6)	BV421	767329	2.5 µL	747915
	CD26	BV480	M-A261	1.25 µL	746696
		BV570			
	CD163	BV605	GHI/61	5 µL	745091
	Axl	BV650	108724	5 µL	747860
		BV711			
	CD36	BV750	CLB-IVC7	0.625 µL	747253
	CD141	BV786	1A4	0.6 µL	741006
Blue 488 nm	HLA-DR	BB515	G46-6	5 µL	564516
		BB630			
		BB660			
	CD3	BB700	SK7 (Leu-4)	5 µL	566575
	CD19	BB700	SJ25C1	5 µL	566396
	CD56	BB700	NCAM16.2 (NCAM16)	5 µL	566573
		BB750			
		BB790			
Yellow-Green 561 nm	CD370 (Clec9A)	PE	3A4/Clec9A	5 µL	563488
	CD14	PE-CF594	MφP9 (MφP-9)	5 µL	562335
		PE-Cy 5			
	CD123	PE-Cy 7	7G3	5 µL	560826
Red 637 nm	CX3CR1	Alexa Fluor™ 647	2A9-1	5 µL	565895
	CD1c	APC-R700	F10/21A3	5 µL	566614
	CD16	APC-H7	3G8	5 µL	560195

BB, BD Horizon Brilliant Blue; BUV, BD Horizon Brilliant UV; BV, BD Horizon Brilliant Violet™. Blank rows for specific fluorochromes indicate additional available channels in the BD FACSymphony A3 System configuration that were not utilized in this study.

Figure 1

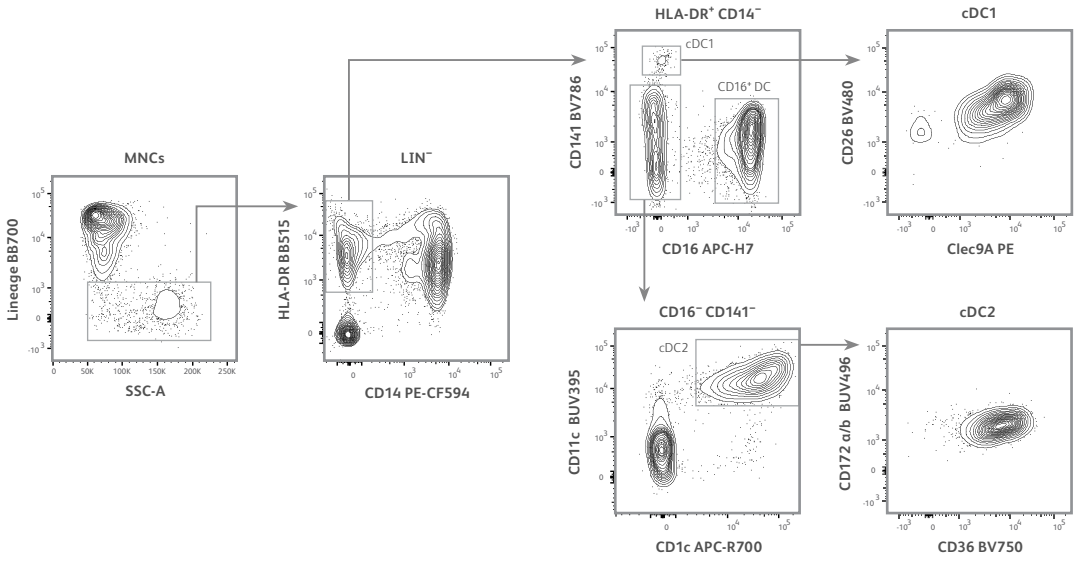

Figure 1. Identification of DC subsets circulating in human peripheral blood

Mononuclear cells (MNCs) were first gated based on light scatter properties and then on singlets (not shown); lineage (CD3,CD56,CD19)$^+$ cells were excluded from analysis. DCs were gated on lineage negative (LIN$^-$) HLA-DR$^+$CD14$^-$ cell population. From (LIN$^-$) HLA-DR$^+$CD14$^-$ cells, the cDC1 subset was identified with the CD141$^+$CD16$^-$ phenotype and shown to express Clec9A and CD26. The cDC2 subset was derived from CD16$^-$CD141$^-$ population and identified by co-expression of markers CD1c and CD11c. The cDC2 population was further analyzed to assess cell surface expression of CD36 and CD172a/b (SIRPα/β1). CD16$^+$CD141$^-$ cells were marked as non-classical DC that express CD86 (not shown).

Figure 2A

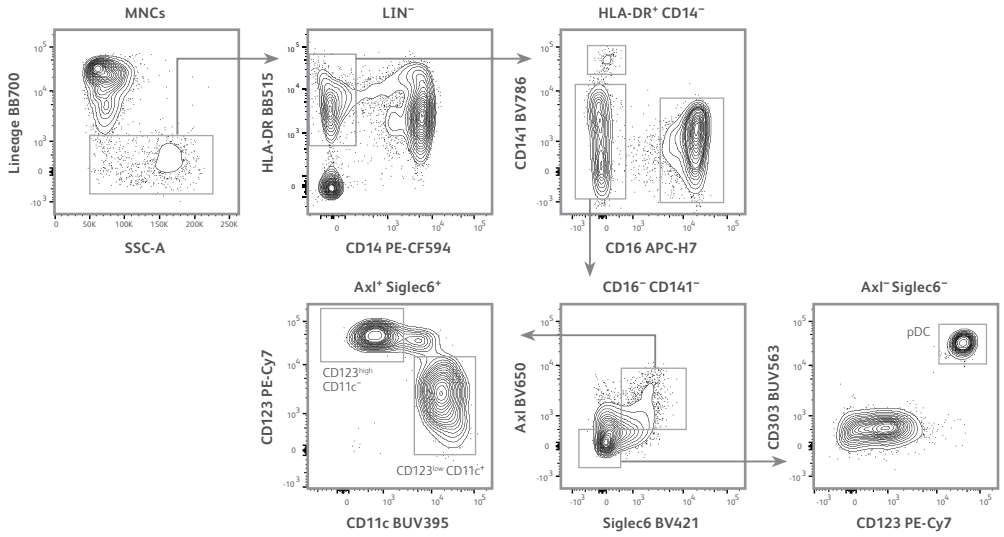

Figure 2B

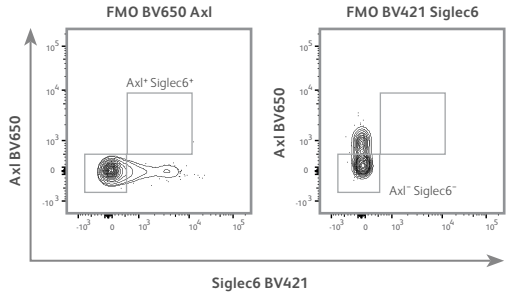

Figure 2. Identification of AS DC and pDC population in human peripheral blood

A. DCs were identified following a gating strategy as described in Figure 1. AS DC subset was gated from CD16⁻CD141⁻ cells and defined based on co-expression of Axl and Siglec6. As shown, two major AS DC subsets CD123^{high} CD11c⁻ and CD123^{low} CD11c^{+} were observed (Villani et al.[2]). The pDC population was gated from the Axl⁻ Siglec6⁻ subset and identified by co-expression of CD123 and CD303. **B.** FMO controls for Axl and Siglec6 are shown, which follow a gating strategy as described in Figure 2A.

Figure 3

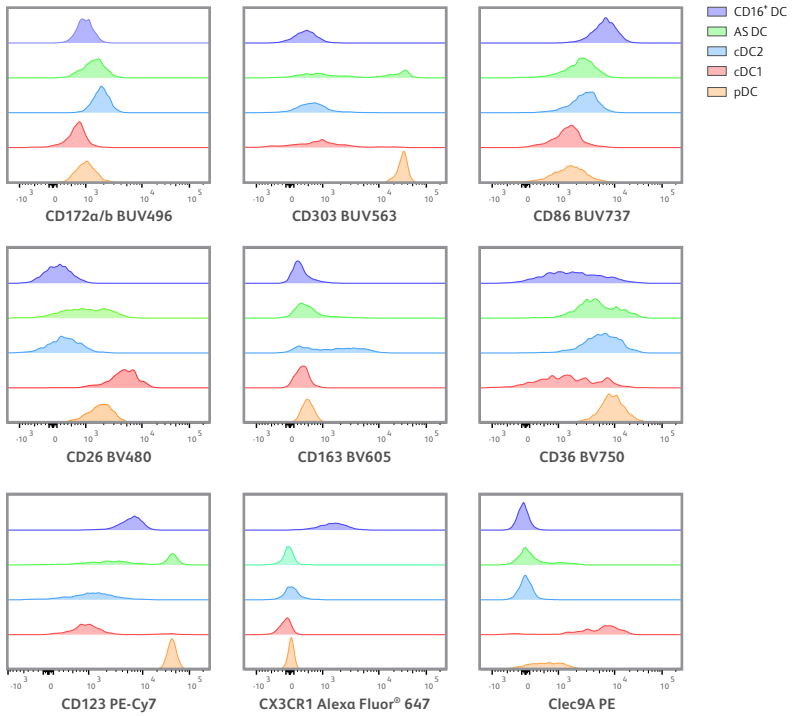

Figure 3. Cell surface marker expression on human DC subsets

Histograms show distribution of selected cell surface markers on human DC identified using multicolor DC panel. DC subsets in healthy donor PBMCs were identified following gating strategy as described previously. Phenotypic marker expression was shown for representative DC subsets (CD16^{+} non-classical DC, Axl^{+}Siglec6^{+} (AS) DC, cDC1, cDC2 and pDC) as histogram overlays.

Figure 4

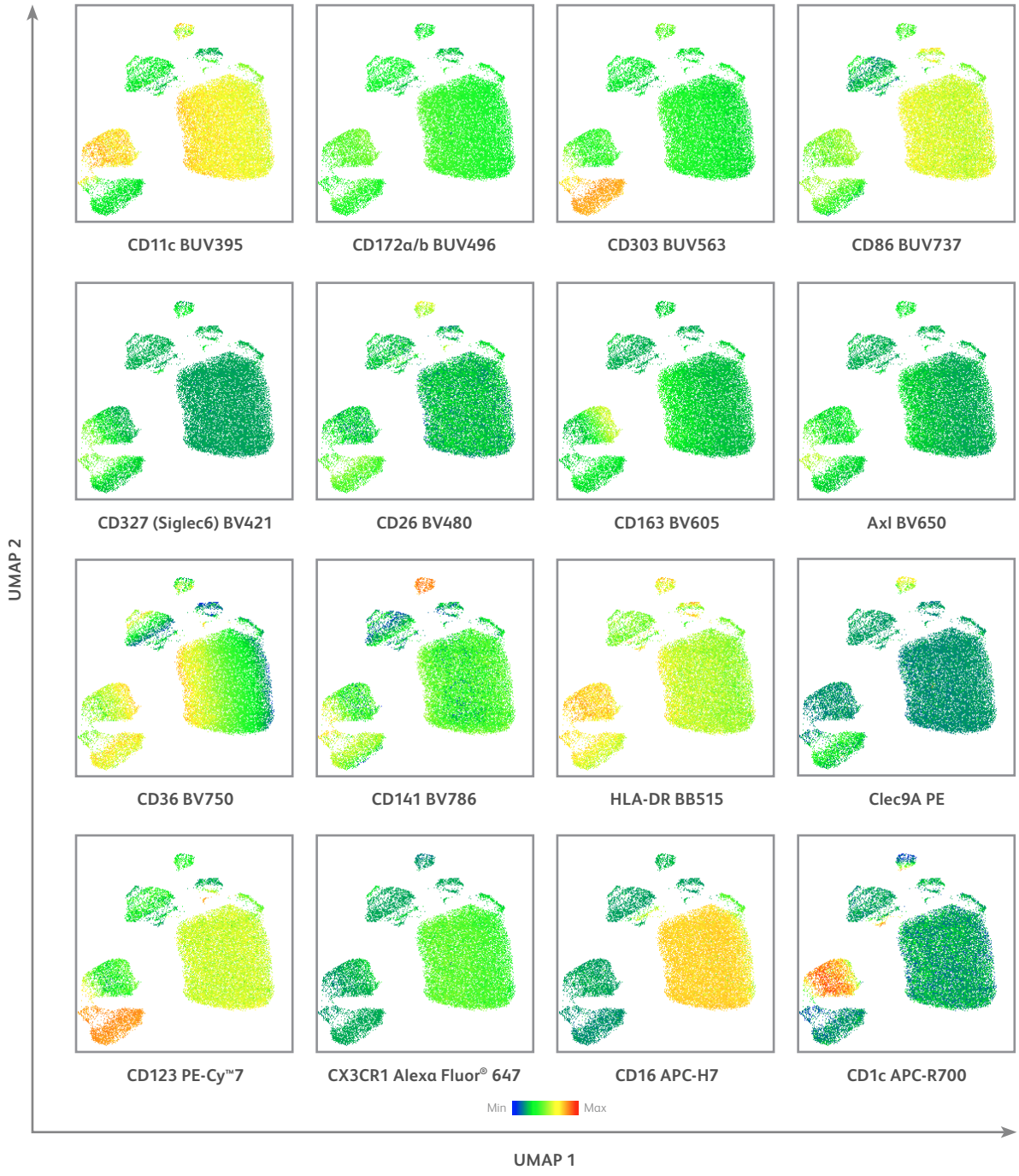

Figure 4. Unsupervised analysis of flow cytometry data for DC marker expression

Data acquired on the BD FACSymphony A3 Cell Analyzer were evaluated by dimensionality reduction algorithm UMAP (FlowJo Software). HLA-DR^{+}CD14^{-} cells were identified following flow cytometry–based manual gating and then analyzed via UMAP plugin defined parameters. Heatmap statistics for individual cell surface markers are shown. Color scale indicates signal intensity of markers.

Conclusion

Data presented here establish performance of a 20 fluorochrome multiparametric human DC panel run on the BD FACSymphony A3 Cell Analyzer. The fluorescent antibody panel enabled identification of major human DC subsets, confirmed subset-specific marker expression and is suitable for both conventional and unsupervised analysis of human DCs. The BD FACSymphony A3 Cell Analyzer offers flexibility and breadth to include 28-color combination for immunophenotyping immune cell populations.

Reference

1. Jongbloed SL, Kassianos AJ, McDonald KJ, et al. Human CD141^{+} (BDCA-3)$^{+}$ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med*. 2010;207(6):1247-1260. doi:10.1084/jem.20092140
2. Villani AC, Satija R, Reynolds G, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science*. 2017;356(6335):eaah4573. doi:10.1126/science.aah4573
3. Collin M, Bigley V. Human dendritic cell subsets: an update. *Immunology*. 2018;154(1):3-20. doi:10.1111/imm.12888

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