Leukocyte immunophenotyping of PBMCs: 17-color flow cytometry panel design using the NovoCyte Quanteon™ flow cytometer

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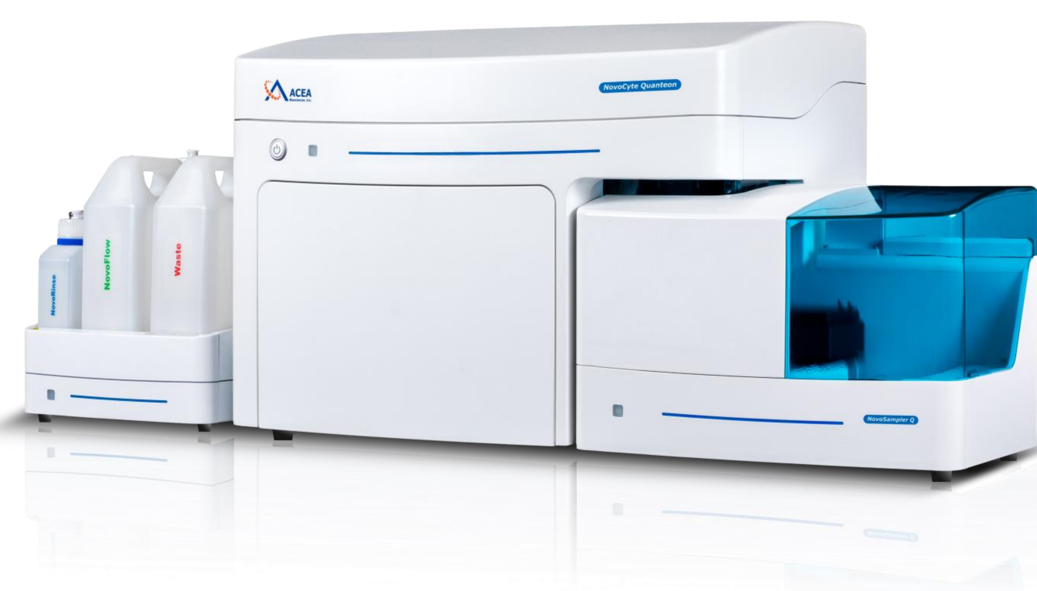
Abstract

Simultaneous quantification of multiple leukocytes allows for better surveillance of the immune response to infectious disease and the immune status patients. With the increasing capabilities of flow cytometers to perform complex multi-color analysis, the opportunity to monitor more immune subsets is made possible. Using the NovoCyte Quanteon flow cytometer, a 17 color panel adapted from OMIP-024 was used to monitor various immune subsets in PBMCs including, monocytes, B cells, plasmablasts, T cells, γδ T cells, NK T cells, NK cells, and dendritic cells. Further analysis of NK and T cell activation and differentiation state were performed to obtain a deeper understanding of the immune status. This information was achieved with a surface staining using 17 antibodies against the following markers: CD3, CD4, CD8, CD19, CD14, CD56, CD16, γδ TCR, Vδ2 TCR, CD25, CD127, CD45RA, CCR7, CD57, HLA-DR, CD38 and NKG2C. With this panel, the phenotype and activation of many cell subtypes can be determined in one run which allows quick and in-depth analysis of human leukocyte subsets. The NovoCyte Quanteon flow cytometer features high resolution of dim signals and flexible configuration and is particularly suitable for multicolor analysis.

Introduction

The human immune system is highly complex and immune status is associated with disease status, treatment efficiency, and response to external stimuli such as vaccines. Monitoring leukocytes has also gained diagnostic importance in the prognosis and prediction of therapies. This study is based on a multi-color flow cytometry panel, OMIP-024 (Moncunill et al. Cytometry Part A,85A:995-998, 2014), originally designed to measure the relative frequencies of different leukocyte subsets in the peripheral blood mononuclear cells (PBMCs) of African children to monitor vaccine efficacy. Therefore it was important to obtain the maximum information concerning the status of the immune system with a small amount of blood. Monitoring numerous immune cell population frequencies and differentiation/activation status of specific cell subsets such as monocytes, NK cells, T and B cells is essential as they may influence the immunogenicity of a vaccine and its efficiency.



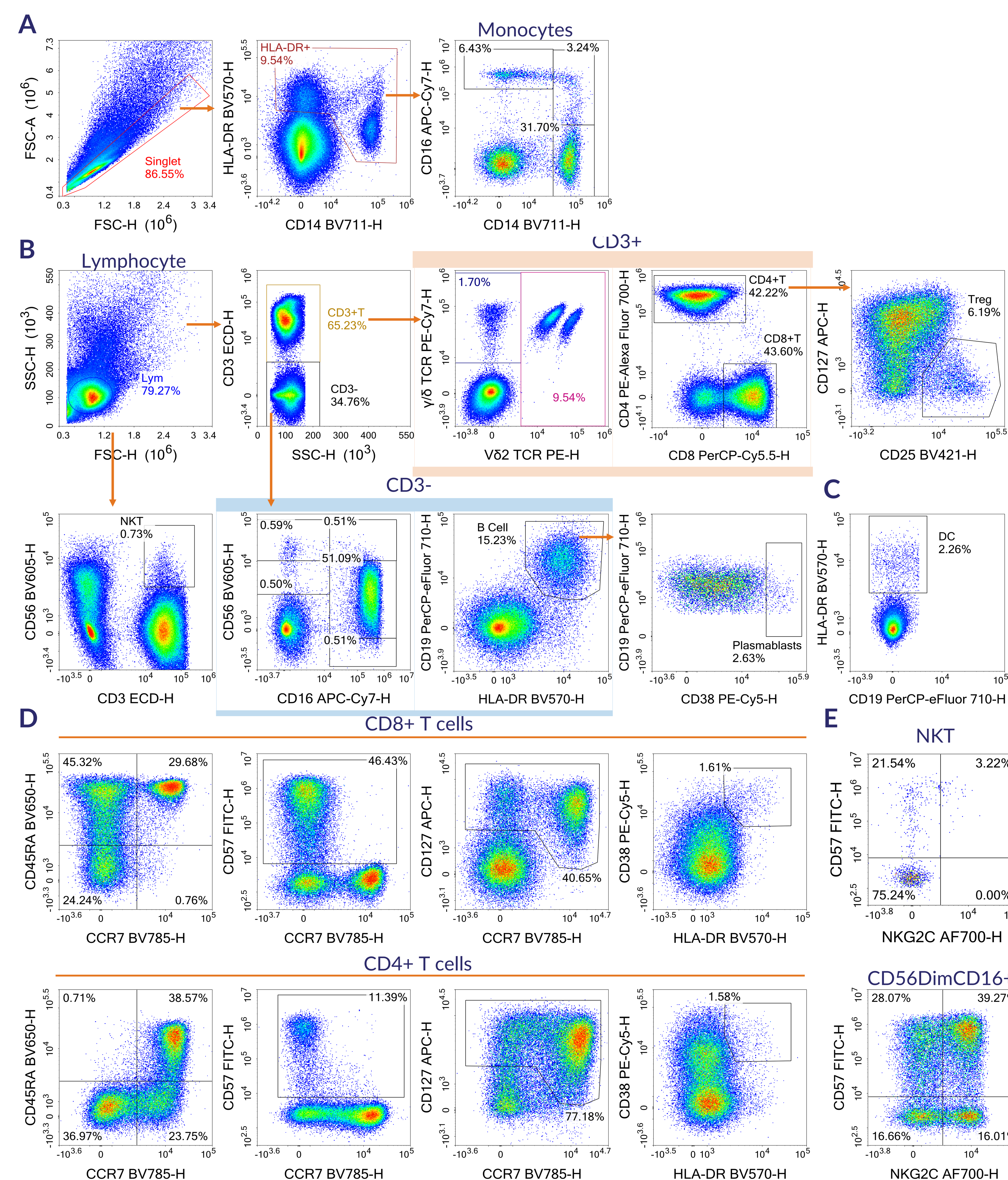
NovoCyte Quanteon™

The NovoCyte Quanteon flow cytometer builds on its successful predecessor. It offers 25 fluorescent channels utilizing 4 lasers to accommodate increasingly sophisticated multi-color flow cytometry assays. Through comprehensive consideration and analysis of the expression of each marker, fluorescence intensity, spectral overlap, and NovoCyte Quanteon configuration, a 17-color immune phenotyping (immunophenotyping) panel was designed for studying human PBMCs using the NovoCyte Quanteon. Here, we examined the frequency of monocytes, B cells, plasmablasts, CD4+ and CD8+ T cells, regulatory T cells, γδ T cells, NK T cells, NK cells, and dendritic cells. Expression of activation and differentiation markers were also examined on NK and T cells

This panel was originally designed to monitor response to a malaria vaccine where NK and γδ T cell responses have been indicated as having particular importance. Therefore, Expression levels of CD56 (neural cell adhesion molecule NCAM) and CD16 (Fcγ IIIa) were used to define five NK subsets, while Vδ2 and γδ TCR were included to identify γδ T cells. Furthermore, NK T cells were identified as CD3+CD56+ cells. Furthermore, activated NK cells were identified by NKG2C staining. Regulatory T cells were identified by the expression of CD25 (IL-2Rα-chain) and CD127 (IL-7Rα-chain) on CD4+ T cells to avoid the need to do intracellular staining for Foxp3. In depth analysis of T cell subsets were achieved by CD45RA and CCR7 co-staining: naïve (CD45RA+CCR7+), central memory (CD45RA-CCR7+), effector memory (CD45RA-CCR7-), and terminal effector memory (CD45RA+CCR7-). Further T cell subset analysis was performed by examining the expression of CD127 (homeostatic proliferation) and CD57 (cell senescence). CD38 and HLA-DR were included to evaluate T cell activation as well as identify plasmablasts (CD19+CD38hi). Dendritic Cells were identified as negative for all lineage markers but positive for HLA-DR. All gating was determined with the use of fluorescence minus one (FMO) controls. This panel allows the simultaneous assessment of multiple immune cell subsets in one sample.

SPECIFICITY	CLONE	FLUOROCROME	PURPOSE
CD3	UCHT1	PE-TR (ECD)	
CD4	S3.6	PE-Alexa 700	Lineage T cells
CD8	SK1	PerCP-Cy5.5	
CD19	J3-129	PerCP-eFluor 710	B cells
CD14	MφP9	BV711	Monocytes
CD56	HCD56	BV605	NK cells and NK T-like cells
CD16	3G8	APC-Cy7	NK cells and monocytes
γδ TCR	11F2	PE-Cy7	γδ T cells
Vδ2 TCR	B6	PE	
CD25	M-A251	BV421	Tregs
CD127	A019D5	APC	Tregs/memory/differentiation
CD45RA	HI100	BV650	
CCR7	G043H7	BV785	Memory/differentiation
CD57	NK-1	FITC	
HLA-DR	B169414	BV570	Activation
CD38	HIT2	PE-Cy5	Activation/plasmablasts
NKG2C	134591	Alexa 700	NK receptor

17 Color Human Pan-Leukocyte Analysis: Gating Strategy



17-color Pan-Leukocyte flow cytometry panel

PBMCs from a normal donor were stained with the 17 color panel stain. **(A)** Initial gating was done on FSC-H and FSC-A to discriminate single cells. Monocytes were identified as HLA-DR+;Three monocyte subsets were identified: classical (CD14+CD16-), intermediate (CD14+CD16+), and non-classical (CD14dimCD16+). **(B)** FSC-H and SSC-H were used to identify lymphocytes. CD3 was used to identify T cells. Subsequent gating of CD3+ cells identified Vδ2+ and Vδ2- γδ T cells as well as CD4+ and CD8+ T cells. Regulatory T cells (Tregs) were identified as CD25hi CD127lo CD4+. NK T cells were identified by the co-expression of the NK marker,CD56, and CD3. Among the CD3- cells, five NK cell subsets were identified by expression of CD56 and CD16 (CD56hiCD16-. CD56dimCD16-, CD56hiCD16+, CD56dimCD16+, and CD56-CD56+). B cells were identified as CD19+HLA-DR+. Plasmablasts were identified within the CD19+ cells as CD38hi. **(C)** Dendritic cells were identified as being negative in all lineage markers but positive for HLA-DR **(D)** Activation status of CD4+ and CD8+ T cells were examined by the expression of CCR7, CD45RA, CD57, CD127, HLA-DR and CD38. **(E)** Activation of NK and NKT cells were examined by CD57 and NKG2C expression. Plots show expression of NKG2C and CD57 in CD56dimCD16+ NK Cells and NKT cells. This panel was adapted from OMIP-024 (Cytometry Part A,85A:995-998, 2014).

Conclusion

Here we demonstrated a 17-color multi-parameter panel on the NovoCyte Quanteon which identifies numerous leukocyte subsets. The cell types identified includes monocytes, B cells, plasmablasts, T cells, γδ T cells, NK T cells, NK cells, and dendritic cells in PBMCs; allowing a broad overview of all leukocytes in only one stain. Complex multi-parameter flow cytometer experiments has been made possible with new high performance, multi-laser flow cytometers such as the NovoCyte Quanteon, a 4 laser 27 parameters instrument. The addition of more parameters to current flow cytometry experiments will expand the capability to understand complex interactions of the numerous cell subsets of the immune system as well as their activation/differentiation status.

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