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Immunophenotyping

Identification of 18 immune cell subsets in human blood using a 13-color panel

Background

Flow cytometry has become the method of choice for immunophenotyping and identifying specific cellular subsets. Within seconds, it provides a thorough overview of the major cell types that constitute a sample. Using multiple markers simultaneously increases the number of parameters that can be analyzed per run and decreases the amount of starting material required to perform an assay. This can be critical for precious sample material and long-term immune-monitoring studies. In this application note, we demonstrate 13-color immunophenotyping of human peripheral blood mononuclear cells (PBMCs) using the MACSQuant® Analyzer 16, a compact and reliable benchtop flow cytometer equipped with three lasers. The markers selected allow for the simultaneous identification and analysis of 18 different cell populations, thus maximizing the amount of information that can be retrieved from the sample material analyzed. This is critical when input material is limited, as is often the case for pediatric or disease studies.

Materials and methods

PBMCs were labeled with CD14-VioBlue®, CD45-VioGreen™, CD8-BV570™, Anti-IgD-BV605™, CD62L-BV650™, CD25-VioBright™ 515, CD27-PE, CD45R0-PE-Vio 615, CD4-PerCP-Vio 700, CD19-PE-Vio 770, CD127-APC, CD3-Alexa Fluor® 700 and CD24-APC-Vio® 770. Data was acquired on the MACSQuant Analyzer 16 using MACSQuantify™ Software for acquisition and Flowlogic™ Software for analysis. The markers used to identify different immune cell populations are described in table 1.

Cell staining protocol

1. Resuspend 2×10^6 leukocytes from lysed whole blood in 100 µL of PEB Buffer (phosphate buffered saline, pH 7.2, 2 mM EDTA, 0.5% BSA).
2. Add conjugated antibodies at vendor recommended concentrations.
3. Incubate for 10 minutes at 4°C.
4. Add 1 mL of PEB Buffer to wash cells.
5. Centrifuge at 300×g for 10 minutes and aspirate supernatant.
6. Resuspend pellet in 500 µL of PEB Buffer.

Cell type	Function	Phenotype
Eosinophils	Parasitic immunity	$CD45^+$, $SSC^{mid/hi}$, $CD14^-$, $CD16^-$, $CD19^-$
Neutrophils	Innate Immunity	$CD45^+$, $SSC^{mid/hi}$, $CD14^-$, $CD16^+$, $CD19^-$
Classical monocytes	Phagocytosis of pathogens and antigen presentation	$CD45^+$, SSC^{mid}, $CD14^+$, $CD16^-$
Intermediate monocytes	Phagocytosis of pathogens and antigen presentation	$CD45^+$, SSC^{mid}, $CD14^+$, $CD16^{mid}$
Non-classical monocytes	Phagocytosis of pathogens and antigen presentation	$CD45^+$, SSC^{mid}, $CD14^+$, $CD16^+$
Class-switched memory B cells	Adaptive immunity	$CD45^+$, SSC^{low}, $CD19^+$, $CD27^+$, IgD^-, $CD14^-$
Non-switched memory B cells	Adaptive immunity	$CD45^+$, SSC^{low}, $CD19^+$, $CD27^+$, IgD^+, $CD14^-$
Naive B cells	Adaptive immunity – non-antigen experienced	$CD45^+$, SSC^{low}, $CD19^+$, $CD27^-$, IgD^+, $CD14^-$
Cytotoxic effector memory T cells	Killing of virally infected or cancer cells	$CD45^+$, SSC^{low}, $CD19^-$, $CD3^+$, $CD4^-$, $CD8^+$, $CD45RO^+$, $CD62L^-$, $CD14^-$
Cytotoxic central memory T cells	Killing of virally infected cells	$CD45^+$, SSC^{low}, $CD19^-$, $CD3^+$, $CD4^-$, $CD8^+$, $CD45RO^+$, $CD62L^+$, $CD14^-$
Cytotoxic effector T cells	Killing of virally infected cells	$CD45^+$, SSC^{low}, $CD19^-$, $CD3^+$, $CD4^-$, $CD8^+$, $CD45RO^-$, $CD62L^-$, $CD14^-$
Cytotoxic naive T cells	Killing of virally infected cells – non-antigen experienced	$CD45^+$, SSC^{low}, $CD19^-$, $CD3^+$, $CD4^-$, $CD8^+$, $CD45RO^-$, $CD62L^+$, $CD14^-$
Effector memory T helper cells	Immune regulation	$CD45^+$, SSC^{low}, $CD19^-$, $CD3^+$, $CD4^+$, $CD8^-$, $CD45RO^+$, $CD62L^-$, $CD14^-$
Central memory T helper cells	Immune regulation	$CD45^+$, SSC^{low}, $CD19^-$, $CD3^+$, $CD4^+$, $CD8^-$, $CD45RO^+$, $CD62L^+$, $CD14^-$

Table 1: Identification and analysis of PBMC subsets. The table shows a selection of surface markers that can be used for a characterization of immune cell subtypes by flow cytometry. Compiled from references 1 and 2.

Cell type	Function	Phenotype
Effector T helper cells	Immune regulation	CD45$^+$, SSC^{low}, CD19$^-$, CD3$^+$, CD4$^+$, CD8$^-$, CD45RO$^-$, CD62L$^-$, CD14$^-$
Naive T helper cells	Immune regulation – non-antigen experienced	CD45$^+$, SSC^{low}, CD19$^-$, CD3$^+$, CD4$^+$, CD8$^-$, CD45RO$^-$, CD62L$^+$, CD14$^-$
Regulatory T cells	Immune suppression and regulation	CD45$^+$, SSC^{low}, CD19$^-$, CD3$^+$, CD4$^+$, CD8$^-$, CD127$^-$, CD25$^+$, CD14$^-$
NK cells	Viral and cancer cell clearance	CD45$^+$, SSC^{low}, CD19$^-$, CD3$^-$, CD14$^-$, CD16$^+$

Table 1 (continued): Identification and analysis of PBMC subsets. The table shows a selection of surface markers that can be used for a characterization of immune cell subtypes by flow cytometry. Compiled from references 1 and 2.

Results

Figure 1 depicts the gating strategy used to identify target cell populations of interest. By utilizing the expanded fluorescence capability of the MACSQuant® Analyzer 16, it is possible to simultaneously characterize the presence of eosinophils, neutrophils, classical monocytes, intermediate monocytes, non-classical monocytes, class-switched memory B cells, non-switched memory B cells, naive B cells, cytotoxic effector memory T cells, cytotoxic central memory T cells, cytotoxic effector T cells, cytotoxic naive T cells, effector memory T helper cells, central memory T helper cells, effector T helper cells, naive T helper cells, regulatory T cells, and NK cells. The data clearly show that the MACSQuant Analyzer 16 enables high-quality flow analysis with a 13-color antibody panel, opening up new possibilities for deeper pheno-typing and immune-monitoring applications.

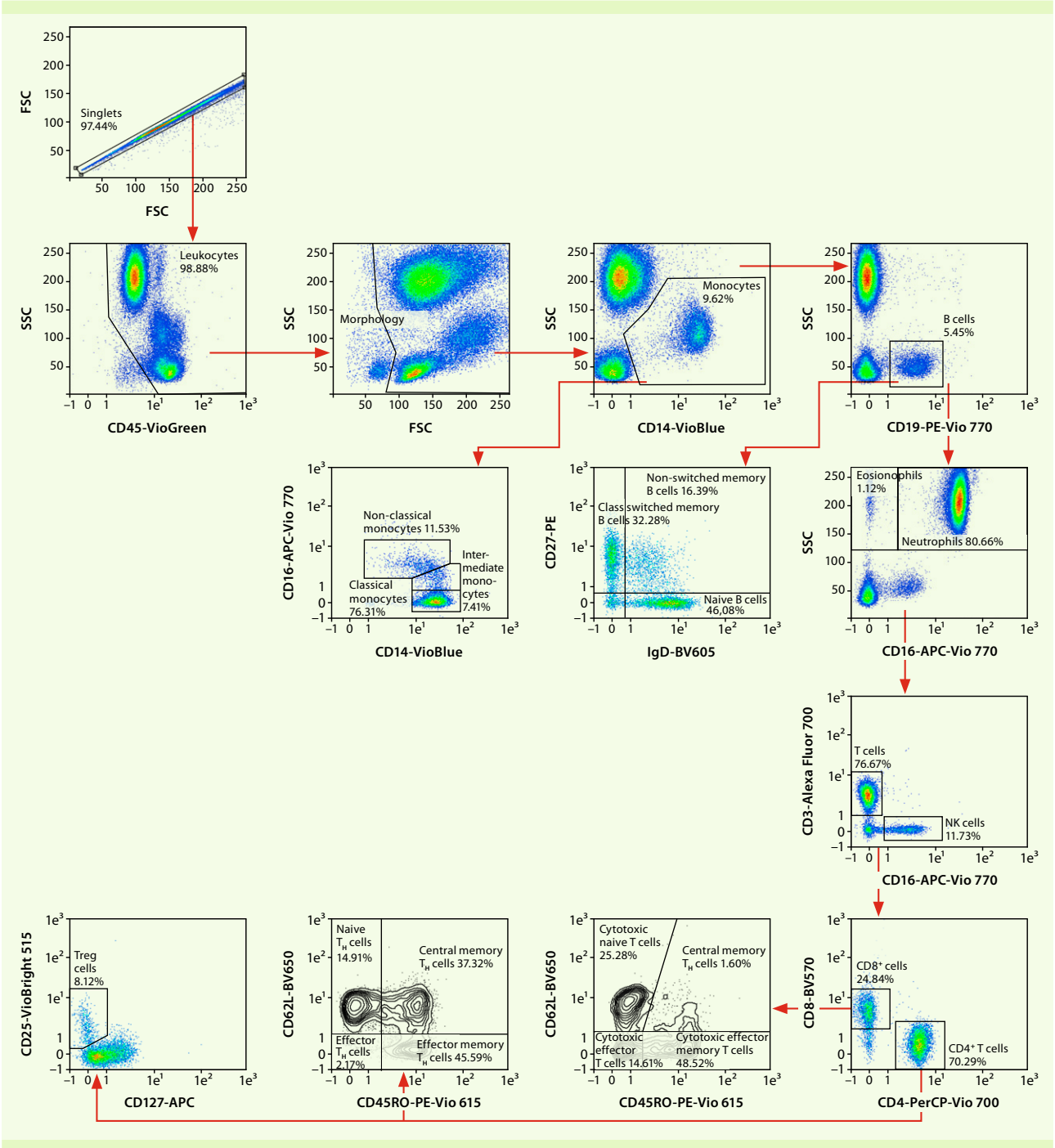

Figure 1: Gating strategy for identification of the target cell populations.

Conclusions

This application note demonstrates the utility of flow cytometry for the detection and enumeration of 18 cellular subsets found in human blood using a 13-color antibody panel.

- Save 50% of sample material by reducing the need to split panels into a second tube/well*
- Use up to 40% less reagents*
- In addition to population statistics, you also gain cell concentration figures for each of the 18 cell subsets analyzed

*compared to an 8-color flow cytometry assay

References

1. Maecker, T.H. *et al.* (2012) Nature Immunol. 12: 191–200.
2. Olingy, C.E. *et al.* (2017) Sci. Rep. 7: 447.

Product	Clone	Order no.
Miltenyi Biotec products		
CD14-VioBlue®	REA599	130-110-524
CD45-VioGreen™	REA747	130-110-638
CD27-PE	M-T271	130-113-630
CD45RO-PE-Vio® 615	REA611	130-113-562
CD4-PerCP-Vio 700	REA623	130-113-228
CD19-PE-Vio 770	REA675	130-113-647
CD127-APC	REA614	130-113-413
CD16-APC-Vio 770	REA423	130-113-390
CD25-VioBright® 515	REA570	Coming soon
Red Blood Cell Lysis Solution		130-094-183
Others		
CD3-Alexa Fluor® 700	OCT3	
CD8-Brilliant Violet 570™	RPA-T8	
IgD-Brilliant Violet 605™	IA6-2	
CD62L-Brilliant Violet 650™	Dreg-56	

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