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Immunophenotyping

Identification of 8 immune cell subsets from mouse spleen using 14-color panel

Background

Flow cytometry has become the method of choice for immunophenotyping and analysis of specific cellular subsets. Using antibodies against selective markers, it provides a quick overview of cell types that constitute a sample. At the same time it allows a thorough analysis on single-cell level for in-depth characterization. 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 a 14-color immunophenotyping of mouse splenocytes allowing for the simultaneous identification and analysis of 8 different immune cell subsets, including viability and activation state of the cells. The data was acquired using the MACSQuant® Analyzer 16, a compact and reliable benchtop flow cytometer equipped with three lasers for high content analysis.

Materials and methods

Splenocytes were labeled with CD11c-VioBlue®, MHCII-VioGreen™, CD11b-BV570™, CD64-BV605™, NK1.1-BV650™, CD172a-Vio® Bright 515, XCR1-PE, CD19-PE-Vio 615, CD3-PerCP, F4/80-PerCP-Vio 700, Siglec-H-PE-Vio 770, CD86-APC, BD Horizon™ Fixable Viability Stain (FVS) 700 and CD45-APC-Vio 770. Data was acquired on the MACSQuant Analyzer 16 using MACSQuantify™ Software . The markers used to identify different immune cell populations are described in table 1.

Cell staining protocol

- Resuspend 2×10^6 splenocytes in 100 µL of PEB Buffer (phosphate buffered saline, pH 7.2, 2 mM EDTA, 0.5% BSA).
- Centrifuge at 300×g for 10 minutes, aspirate supernatant completely and resuspend cells in 100 µL of PBS. Repeat washing step once.
- Stain with Fixable Viability Stain (FVS) according to the manufacturer's instructions.
- Resuspend cells in 100 µL PEB buffer.
- Stain cells with conjugated antibodies according to the manufacturer's instructions.
- Add 1 mL of PEB Buffer and centrifuge at 300×g for 10 min and aspire supernatant. Repeat washing step once.
- Resuspend pellet in 500 µl of PEB Buffer.

Cell type	Function	Phenotype
B cells	Antibody production and adaptive immunity	$CD45^+$, $F4/80^-$, $CD64^-$, $CD3^-$, $NK1.1^-$, $CD19^+$
NK cells	Viral and cancer cell clearance	$CD45^+$, $F4/80^-$, $CD64^-$, $CD3^-$, $NK1.1^+$
NK-T cells	Viral and cancer immunity	$CD45^+$, $F4/80^-$, $CD64^-$, $CD3^+$, $NK1.1^+$
T cells	Immune regulation	$CD45^+$, $F4/80^-$, $CD64^-$, $CD3^+$, $NK1.1^-$
Macrophages	Phagocytosis and innate immunity	$CD45^+$, $F4/80^+$, $CD64^+$
cDC1	Adaptive immunity; activation of $CD8^+$ T cells	$CD45^+$, $F4/80^-$, $CD64^-$, $CD3^-$, $NK1.1^-$, $CD19^-$, $MHCII^+$, $CD11c^+$, $CD172a^-$, $XCR1^+$
cDC2	Humoral immunity; activation of $CD4^+$ T cells	$CD45^+$, $F4/80^-$, $CD64^-$, $CD3^-$, $NK1.1^-$, $CD19^-$, $MHCII^+$, $CD11c^+$, $CD172a^+$, $XCR1^-$
pDC	Viral immunity	$CD45^+$, $F4/80^-$, $CD64^-$, $CD3^-$, $NK1.1^-$, $CD19^-$, $MHCII^+$, $SiglecH^+$, $CD11b^-$

Table 1: Identification of different immune cell subsets in murine splenocytes using flow cytometry. Surface markers for characterization compiled from references 1, 2 and 3.

Results

Figure 1 depicts the gating strategy applied to identify the immune cell subsets of interest. By utilizing the expanded fluorescence capability of the MACSQuant Analyzer 16, it is possible to simultaneously detect the presence of T cell, B cells, NK cells, NK-T cells, macrophages, conventional dendritic cells (cDCs) lineage 1, cDCs lineage 2 and plasmacytoid DCs (pDCs), as well as to screen for cell viability.

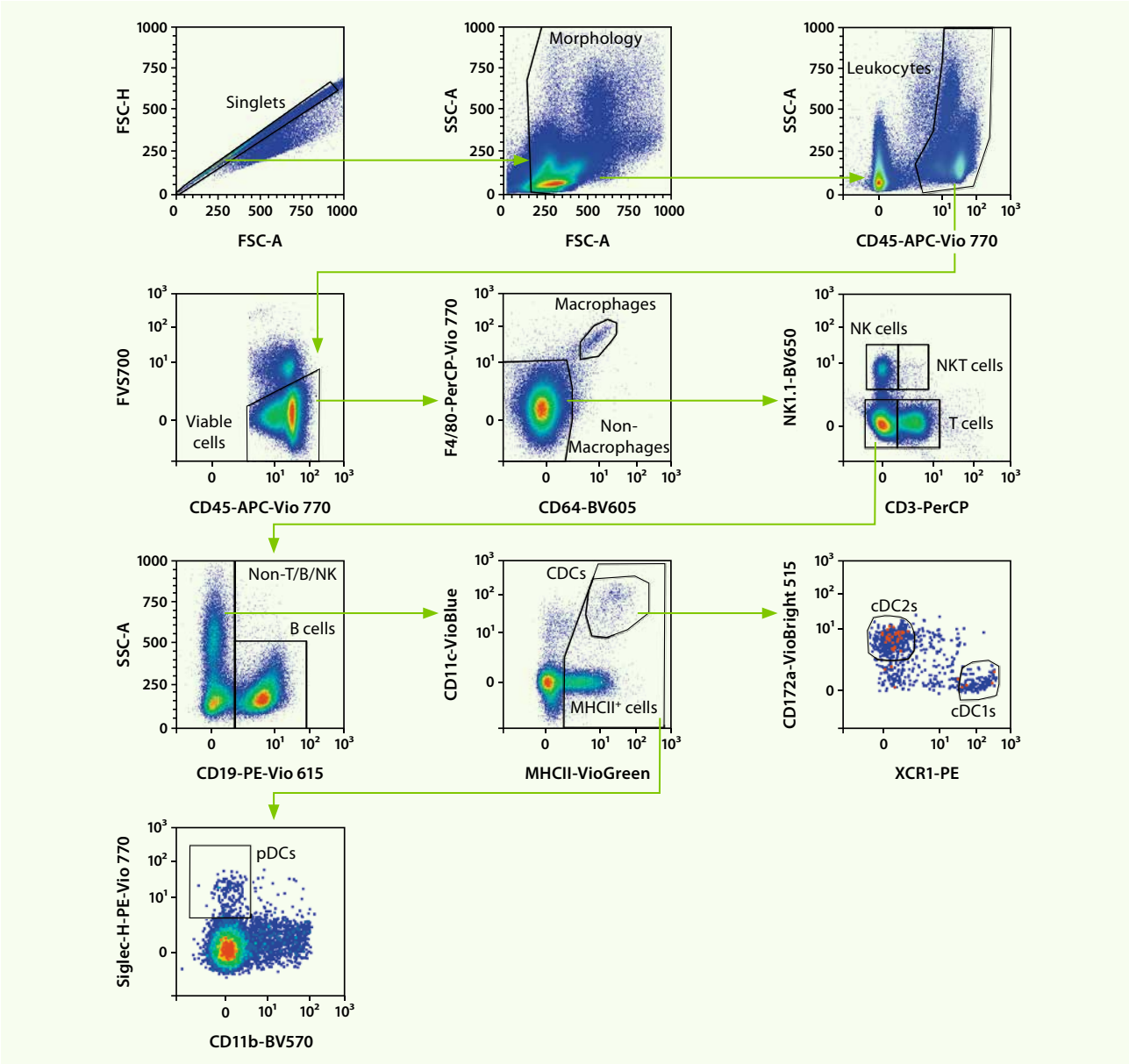

Figure 1: Immunophenotyping of mouse splenocytes. After doublet and dead cell exclusion, further gating was used to identify macrophages, T cells, NK cell, NKT cells, B cells, cDCs1, dDCs2 and pDCs.

Figure 2 shows the evaluation of the expression of the activation marker CD86 on all the above-identified subsets. The data clearly show, that the MACSQuant Analyzer 16 enables high quality flow cytometry data acquisition with a 14-color panel, opening new possibilities for deeper phenotyping and immune monitoring studies.

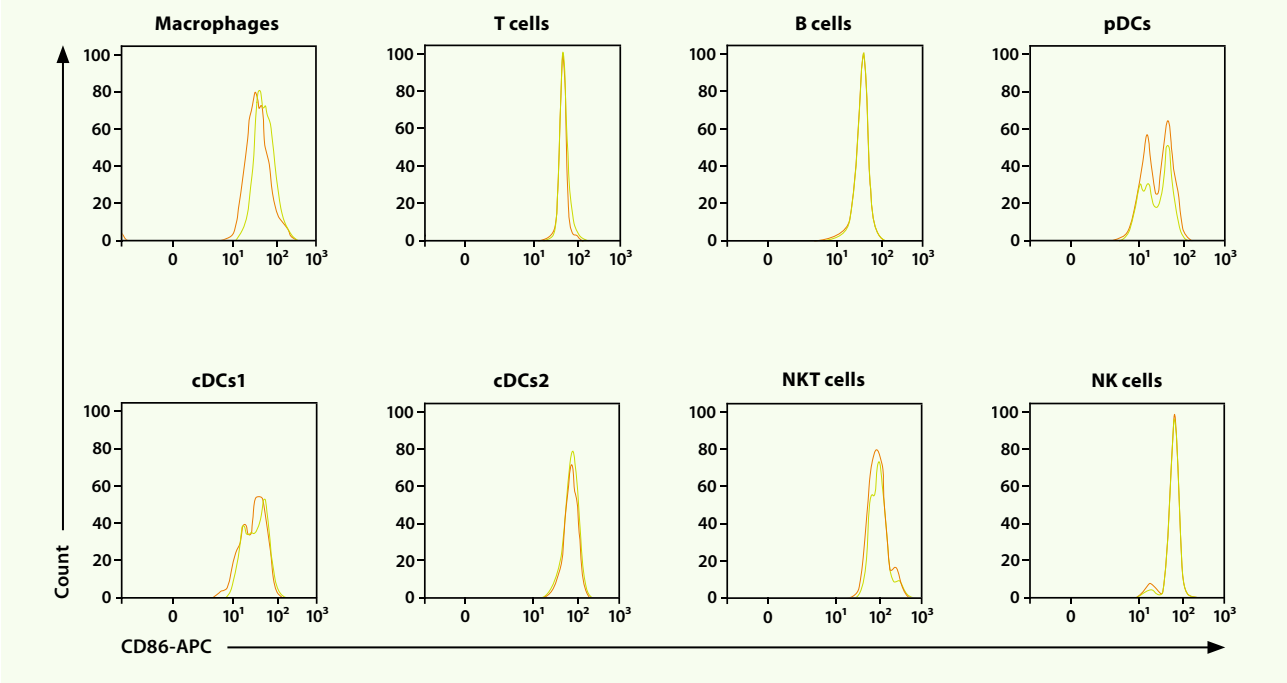

Figure 2: Expression of CD86 on different immune cell subsets. Staining for the cell activation marker CD86 (green) or appropriate control (Fluorescence minus one (FMO), red) on the different subsets identified in figure 1. Cave: staining performed on freshly isolated, unstimulated splenocytes.

Conclusion

This application note demonstrates the utility of flow cytometry for the detection and analysis of 8 immune cell subsets simultaneously including viability and activation status of the identified cells using a 14-color panel. It highlights:

- The possibility to design flow panels with up to 14 colors for the identification of different cellular subsets and for in-depth characterization of cells
- The advantage of high-content flow cytometric analyses when dealing with precious sample material
- The MACSQuant Analyzer 16 as a compact benchtop flow cytometer applicable for advanced immunophenotyping panels of murine cells

References

1. Martens *et al.* (2020) Nat. Immunol. 21, 381-387.
2. Gurka *et al.* (2015) Front. Immunol. 4;6:35.
3. Takagi *et al.* (2011) Immunity 35, 958-971

Product	Clone	Order no.
Miltenyi Biotec products		
CD11c Antibody, anti-mouse, VioBlue, REAfinity™	REA754	130-110-706
MHC Class II Antibody, anti-mouse, VioGreen, REAfinity	REA813	130-112-238
CD172a (SIRPα) Antibody, anti-mouse, Vio Bright 515, REAfinity	REA1201	130-123-157
XCR1 Antibody, anti-mouse/rat, PE, REAfinity	REA707	130-111-184
CD19 Antibody, anti-mouse, PE-Vio 615, REAfinity™	REA749	130-111-890
F4/80 Antibody, anti-mouse, PerCP-Vio 770, REAfinity	REA126	130-118-327
Siglec-H Antibody, anti-mouse, PE-Vio 770, REAfinity	REA819	130-112-140
CD86 Antibody, anti-mouse, APC, REAfinity	REA1190	130-122-130
CD45 Antibody, anti-mouse, APC-Vio 770, REAfinity	REA737	130-110-662
Others		
Brilliant Violet 570 anti-mouse/ human CD11b Antibody	M1/70	-
Brilliant Violet 605 anti-mouse CD64 Antibody	X54-5/7.1	-
Brilliant Voilet 650 anti-mouse NK1.1 Antibody	PK136	-
PerCP anti-mouse CD3ε Antibody	145-2C11	-
BD Horizon™ Fixable Viability Stain (FVS) 700	N/A	-

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