Multicolor immunophenotyping of human and mouse leukocyte subsets using the novel MACSQuant® Analyzer 16

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Introduction

The demand for analyzing multiple parameters in flow cytometry in order to characterize numerous cellular subsets is driven by immunologist since the late 1990's. Meanwhile, multicolor phenotyping of leukocytes by flow cytometry has emerged as the most important application in immunology. It serves as a valuable source of data, both in basic research as well as in the clinical setting. Especially when only limited amount of sample material is available or frequencies of target cells are low, high dimensional flow cytometry is of great advantage.

Here we describe the usage of a novel compact benchtop flow cytometer, the MACSQuant® Analyzer 16, for immunophenotyping of heterogeneous leukocyte subsets. This instrument is equipped with three lasers (405nm, 488nm, 640nm), enabling the detection of 16 optical parameters (fig. 1). In addition, it provides all known features of the MACSQuant® Technology, such as volumetric cell counting, autolabeling, automated plate processing, and an integrated magnetic pre-enrichment unit. To show the capabilities of the MACSQuant® Analyzer 16, two representative phenotyping applications for both murine and human cells were chosen.

MACSQuant Analyzer 16 optical bench

405 nm

405/10 nm	SSC
450/50 nm	VioBlue®
525/50 nm	VioGreen™
579/34 nm	BV 570™
615/20 nm	BV 605™
667/30 nm	BV 650™

488 nm

488/10 nm	FSC
525/50 nm	FITC
579/34 nm	PE
615/20 nm	PE-Vio® 615
667/30 nm	PerCP
725/40 nm	PerCP-Vio® 700
785/62 nm	PE-Vio® 770

640 nm

667/30 nm	APC
725/40 nm	Alexa Fluor® 700
785/62 nm	APC-Vio® 770

Figure 1

Results

1 Immunophenotyping of murine splenocytes

Specificity	Fluorochrome	AB clone	Purpose
Dead cells	Vio 770	n/a	Dump
CD3ε	FITC	17A2	T cell lineage
CD4	VioBlue	REA604	T_H
CD8	Brilliant Violet 570	53-6.7	CTL
CD11b	PE	REA592	Myeloid cells
CD11c	APC	REA754	Dendritic cells
CD19	PE-Vio 615	REA749	B cells
Ly-6C	Brilliant Violet 605	HK1.4	Monocytes
Ly-6G	PerCP-Vio 700	REA526	Neutrophils
MHC class II	VioGreen	REA813	Dendritic cells
NK1.1	Brilliant Violet 650	PK136	NK cells
Siglec-F	APC-R700	E50-2440	Eosinophils
XCR1	PE-Vio 770	REA707	mDC 1

Table 1

For immunophenotyping of murine leukocytes, splenocyte cell suspensions were prepared from C57BL/6 mouse spleen. Isolated cells were stained with Viobility™ Fixable Dye for dead cell exclusion and subsequently labelled with antibodies against different specificities (table 1).

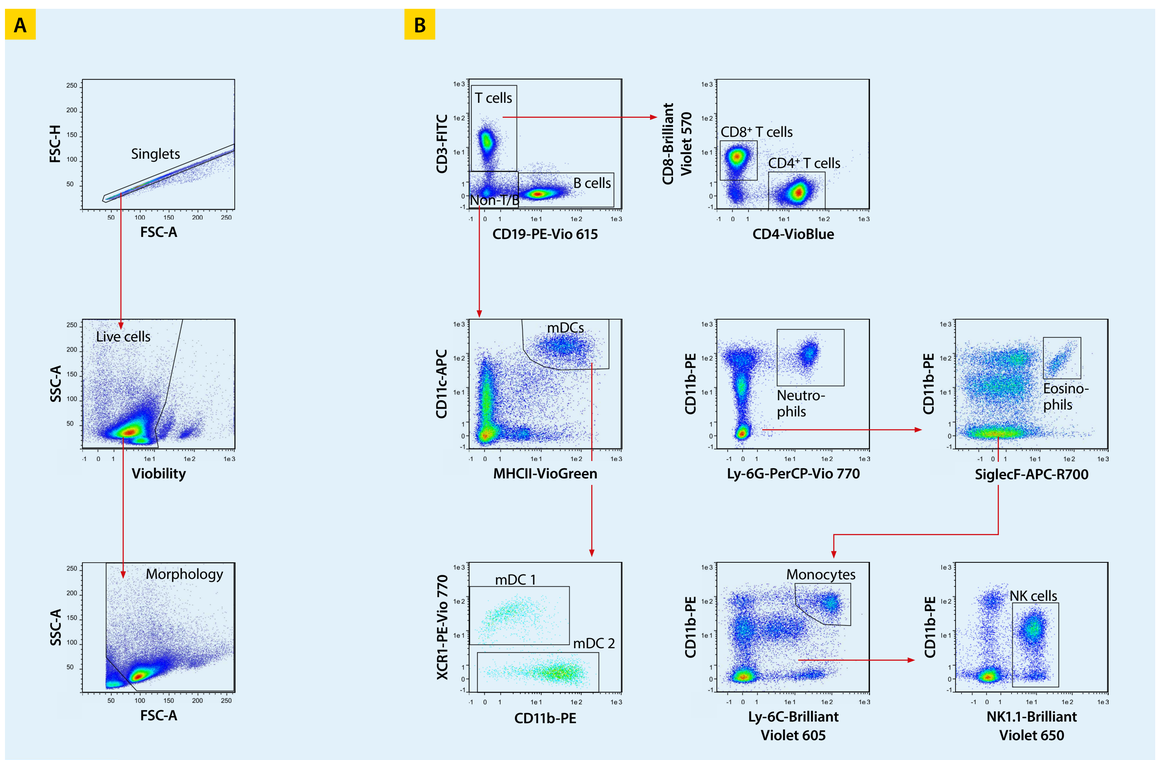

Figure 2

After gating on FSC/SSC, singlets, and living cells (fig. 2A), T and B cells were identified as $CD3^+$ and $CD19^+$, respectively. T cells were further separated into $CD8^+$ cytotoxic T cells (CTL) and $CD4^+$ T helper (T_H) cells (fig. 1A). Remaining cells (labeled as Non-T/B), including dendritic cells (DCs), natural killer cells (NK cells), monocytes, and granulocytes, were further characterized (fig. 2B). First, staining of MHCII and CD11c identified myeloid DCs, which could be further discriminated into mDC 1 and mDC 2 cells, according to their expression of XCR1 and CD11b. Within the non-mDC population, neutrophils were identified as $CD11b^{high}$ $Ly\text{-}6G^+$, whereas CD11b and Siglec-F expression denoted eosinophils. Among the remaining cells, monocytes could be distinguished as $Ly\text{-}6C^{high}$ $CD11b^{high}$, whereas NK cells were $CD11b^{dim/low}$ $NK1.1^+$.

2 Analysis of human blood-derived T cells and chimeric antigen receptor (CAR) T cells

Specificity	Fluorochrome	AB clone	Purpose
Dead cells	7-AAD	n/a	Dump
CD3	VioGreen	REA613	T cell lineage
CD4	Alexa Fluor 700	REA623	T_H
CD8	Brilliant Violet 570	RPA-T8	CTL
CD27	FITC	REA499	Differentiation
CD45RA	PE-Vio 770	REA562	Differentiation
CD45RO	APC-Vio 770	REA611	Differentiation
CD62L	Brilliant Violet 650	DREG-56	Differentiation
CD95	APC	REA738	Differentiation
CD197	VioBlue	REA546	Differentiation
CD19-CAR	PE	n/a	CAR detection

Table 2

To establish a strategy for identification of adoptively transferred CAR T cells in human blood, cultured T cells were engineered to express a CD19-specific CAR and spiked into lysed whole blood of a healthy donor. Subsequently, T cells and CAR T cells were labelled with the panel shown in table 2.

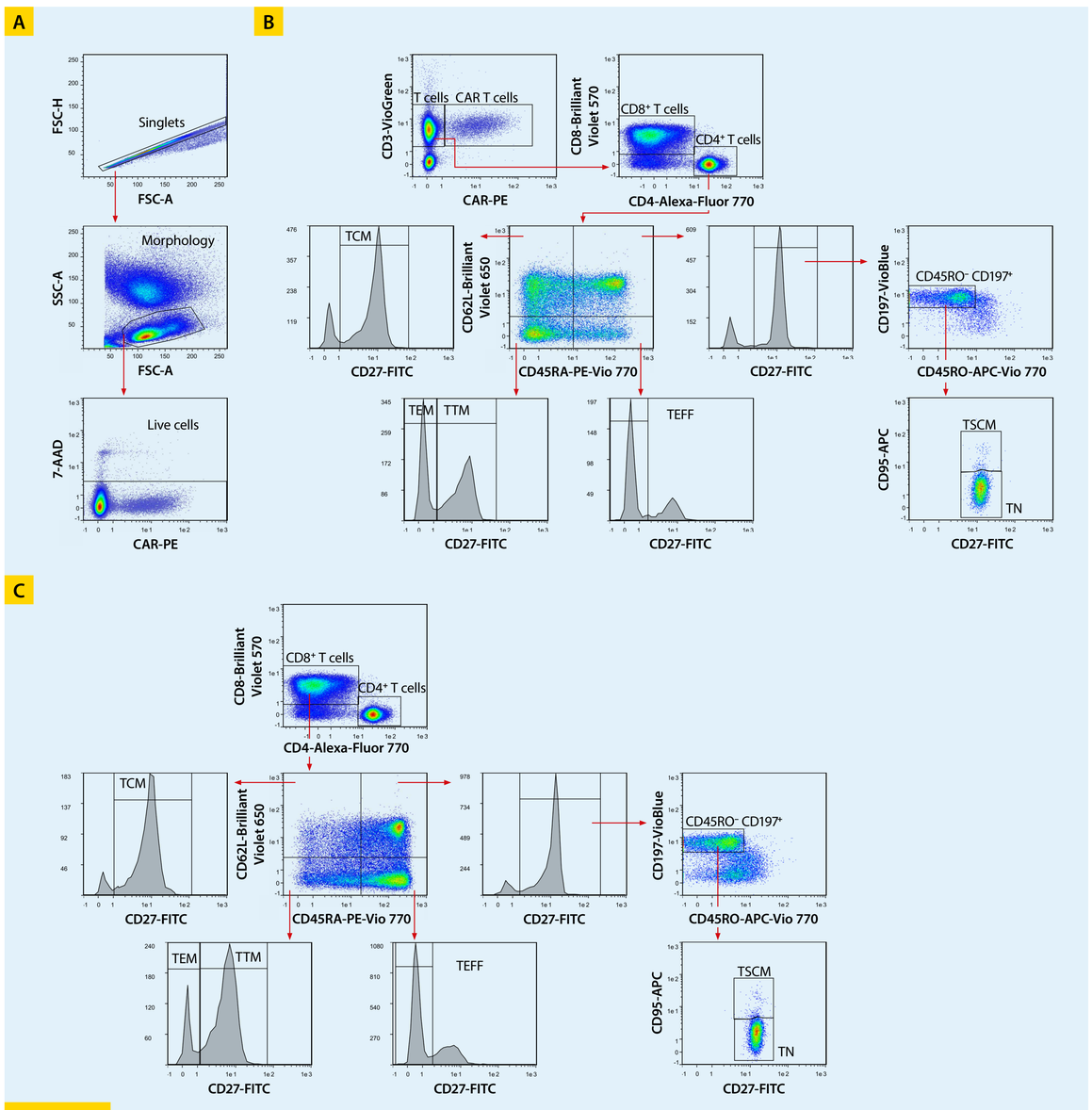

Figure 3

After gating on single, viable lymphocytes (fig. 3A), $CD3^+$ cells were divided into CAR^+ and CAR^- T cells (fig. 3A, top left). For further characterization of blood-derived CAR T cells, $CD4^+$ T_H cells and $CD8^+$ CTLs were discriminated. $CD4^+$ T cells were separated into four subpopulations based on their CD45RA and CD62L expression (fig. 3B, center), which were then further specified according to CD27 density[1]: central memory T cells (TCM) as $CD45RA^-$ $CD62L^+$ $CD27^+$, effector memory T cells (TEM) as $CD45RA^-$ $CD62L^-$ $CD27^-$, transitional memory T cells (TTM), as $CD45RA^-CD62L^-CD27^+$, and effector T cells (TEFF) as $CD45RA^+$ $CD62L^-$ $CD27^-$. For discrimination of stem memory T cells (TSCM), $CD45RA^+$ $CD62L^+$ $CD27^+$ cells were further analyzed.[2] TSCM were identified as $CD197^+$ $CD45RO^-$ $CD95^+$, whereas naïve T cells (TN) were $CD197^+$ $CD45RO^+$ $CD95^-$. The same gating strategy was applied for $CD8^+$ CTLs (fig. 3C).

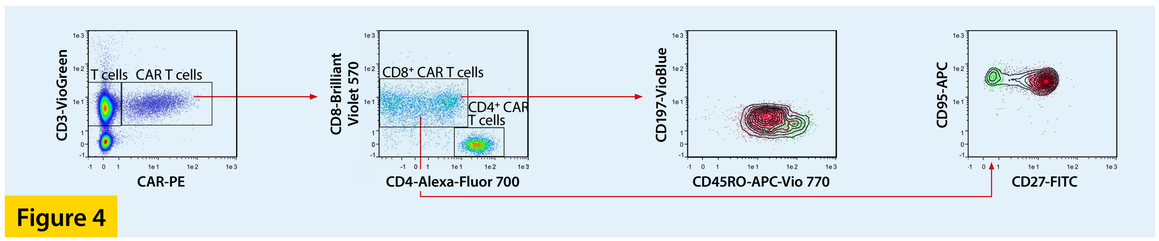

Figure 4

CD19 CAR T cells were identified by using the CD19 CAR Detection Reagent (fig. 4). These cells can be further analyzed with the antibody panel used here (table 2). As an example, two separate populations of $CD8^+$ CAR T cells were distinguished based on their expression of CD45RO, CD197, CD27 and CD95.[3]

Conclusion and outlook

- The MACSQuant® Analyzer 16, a novel compact benchtop flow cytometer, is applicable for advanced immunophenotyping panels of human and murine cells.
- The possibility to design flow panels with up to 14 colors allows for the analysis of complex cellular subsets and is especially useful when sample volume is limited.
- This flow cytometer combines the ease of use and all known features of previous MACSQuant Instrument versions with the option for high content analysis.

References
1. Mahnke, YD *et al.* (2012) Cytometry A 81: 935–936.
2. Gattinoni, L *et al.* (2016) Nat Med 23: 18–27.
3. Sabatino, M *et al.* (2016) Blood 128: 519–528.