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Flow cytometry analysis of Tн subsets

Background

T cells are a heterogeneous cell population comprising different subsets that exert distinct roles in cell-mediated immunity. T cells are commonly subdivided into three major groups, regulatory T (Treg) cells, helper T (Tн) cells, and cytotoxic T (Tc) cells. Tн and Tc cells are characterized by their surface expression of CD4 and CD8, respectively. Activated CD4^{+} Tн cells differentiate into distinct lineages with characteristic patterns.[1–4]

The range of mechanisms used by Tн cells to eliminate pathogens is as wide as the diversity of pathogens. The underlying functional heterogeneity of Tн cells is reflected in distinct patterns of i) cytokine production, ii) expression of surface markers, such as chemokine receptors, and iii) transcription factors involved in regulation. All these characteristics can be used to identify the various Tн cell subsets (table 1). Multicolor flow cytometry is an easy method to distinguish the different Tн subtypes based on these criteria. Here we will focus on the analysis of cell surface markers, including CD molecules and chemokine receptors, which enable a basic characterization of Tн cell subsets.

Materials and methods

Human PBMCs were labeled with CD3 VioBlue®, CD8-VioGreen™, Anti-CCR10-PE, CD183 (CXCR3)-PE-Vio770™, CD194 (CCR4)-APC-Vio770, and CD196 (CCR6)-APC (all from Miltenyi Biotec) and analyzed by flow cytometry on the MACSQuant® Analyzer 10 (Miltenyi Biotec). Dead cells were excluded by propidium iodide fluorescence.

Results and conclusion

Figure 1 depicts a gating strategy to distinguish the different T helper subsets by flow cytometry. Starting from the identification of CD4^{+} T cells, the strategy allows for the characterization of Tн1, Tн2, Tн9, Tн17, and Tн22 cells. Based on this gating strategy a template was developed for the automated analysis on MACSQuant Flow Cytometers. A screenshot of the analysis performed with the MACSQuantify™ Software is shown in figure 2. The template is available for **download on the Miltenyi Biotec website**. However, the gating strategy can also be adapted easily on any other flow cytometer.

Tн cell subset	Major function	Surface markers	Cytokines secreted	Transcription factors involved in regulation
Tн1	Immunity against intracellular pathogens	CD4^{+}CXCR3^{+}	IFN-γ	T-bet
Tн2	Response to parasite infections	CD4^{+}CCR4^{+}CCR6^{−}	IL-4, IL-5, IL-13	STAT6, GATA3
Tн9	Response to parasite infections and large extracellular pathogens	CD4^{+}CCR4^{−}CCR6^{+}	IL-9	IRF-4
Tн17	Response to fungi and extracellular bacteria	CD4^{+}CCR4^{+}CCR6^{+}	IL-17A, IL-22	RORγt
Tн22	Potentially contribute to host defense against microbial infections in the skin	CD4^{+}CCR4^{+}CCR6^{+}CCR10^{+}	IL-13, IL-22	BNC2, FOXO4

Table 1: Features of Tн cell subsets. The table shows a selection of surface markers, cytokines, and transcription factors, which can be used for a basic characterization by flow cytometry. Compiled from references 1–4.
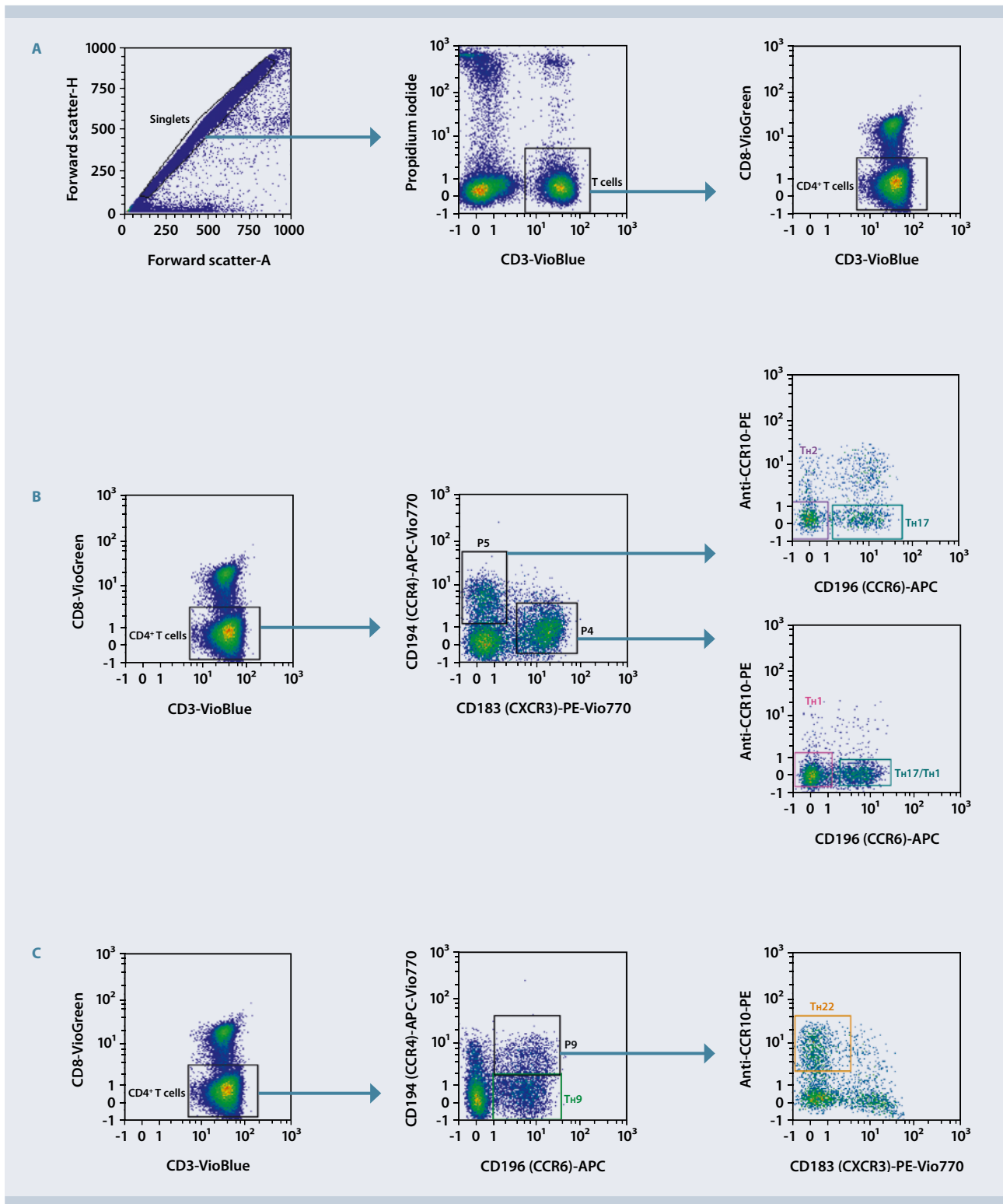
Figure 1: Gating strategy for the identification of TH1, TH2, TH9, TH17, and TH22 cells. (A) Singlets were identified by FSC-A vs. FSC-H blots (A, left). After gating on live T cells (PI⁻CD3⁺; A, middle), CD4⁺ TH cells were identified by excluding CD8⁺ Tc cells (A, right). **(B)** The CD4⁺ cells (B, left) were further analyzed for CXCR3 and CCR4 expression (B, middle). TH2 and TH1 cells could then be distinguished from TH17 cells by CCR6/CCR10 analysis (B, right). **(C)** The CD4⁺ cells (C, left) were also analyzed for CCR6 and CCR4 expression to identify TH9 cells (C, middle). TH22 cells were then detected by CXCR3/CCR10 analysis (C, right).
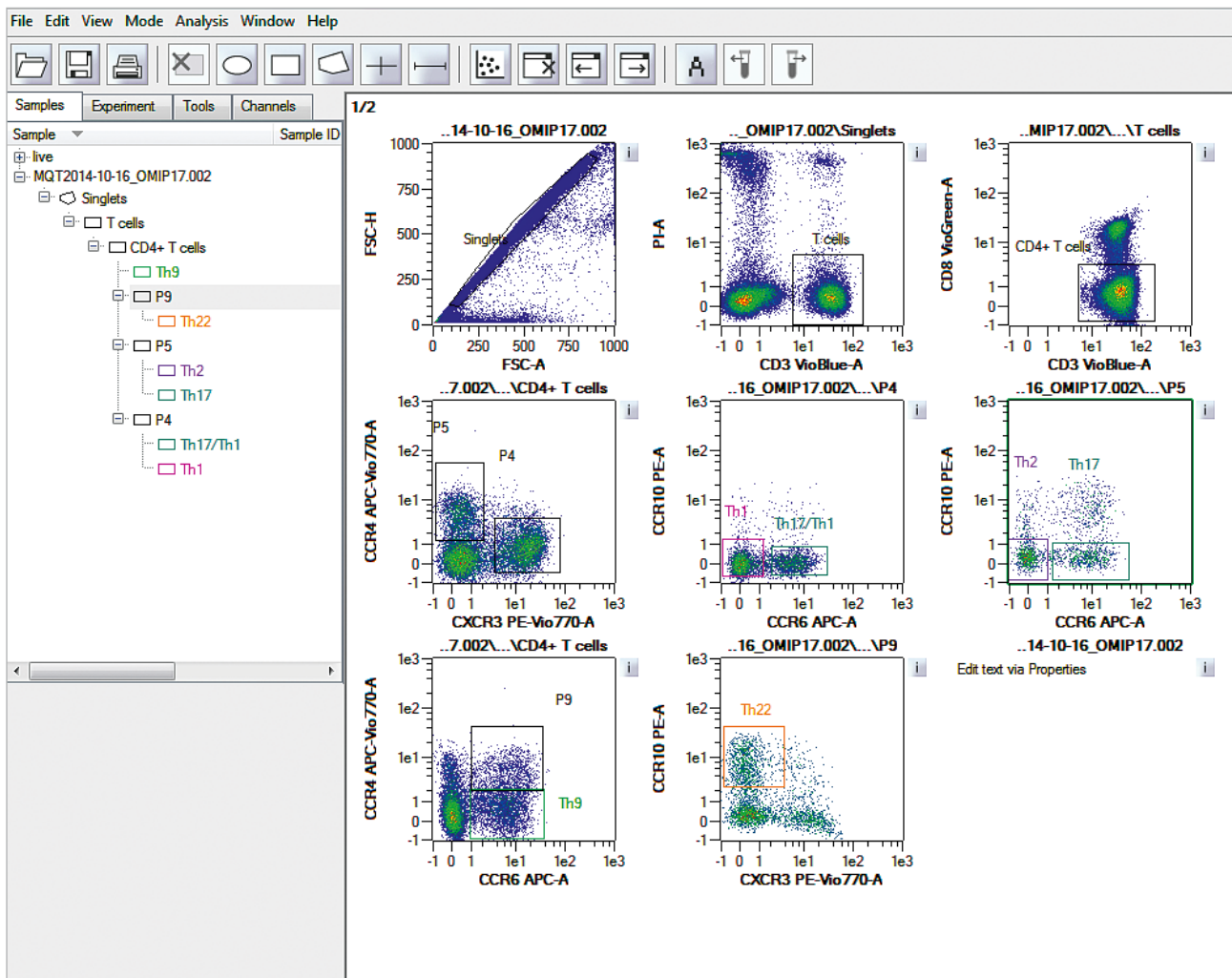
Figure 2: Gating strategy for the identification of Tн cell subsets. Screenshot of the template for the automated analysis of Tн cell subsets on the MACSQuant Analyzer 10.

References

1. Duhen, T. *et al.* (2012) Functionally distinct subsets of human FOXP3⁺ Treg cells that phenotypically mirror effector Tн cells. Blood 119: 4430–4440.
2. Luckheeram, R. *et al.* (2012) CD4⁺ T cells: Differentiation and functions. Clin. Dev. Immunol. 2012: 925135.
3. Mahnke, Y. *et al.* (2013) OMIP-017: Human CD4⁺ helper T-cell subsets including follicular helper cells. Cytometry A 83: 439–440.
4. Eyerich, S. *et al.* (2009) Tн22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. J. Clin. Invest. 119: 3573–3585.

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