CD4$^+$ T Cell Subset Panel

Multicolor flow cytometry panel for the detection of major human circulating CD4$^+$ T cell subsets

The CD4$^+$ T cell subset panel is a 12-color panel designed for the resolution of multiple well-defined and widely studied subsets of circulating human CD4$^+$ T cells. Several chemokine receptors (CXCR3, CXCR5, CCR4 and CCR6) are included in the panel. These markers are routinely used as surface marker surrogates for intracellular transcription factors and cytokines that identify the different subpopulations of circulating human CD4$^+$ T cells. A gating strategy adapted from seminal studies[1-3] was used to identify subsets of conventional memory T helper cells (Th1, Th2, Th17, Th22, Th9 and ThG), T follicular helper cells (Tfh1, Tfh2 and Tfh17), regulatory T cells (Tregs) (Th1-, Th2-, Th17- and Th22-like), as well as T follicular regulatory cells (Tfr). An imbalance between some of these subsets has been described in the context of autoimmunity, viral infection and cancer.

Table 1. Instrument configuration and reagent selection

Laser Line	Marker	Fluorochrome	Clone	Volume Per Test	Catalog Number
Violet 405 nm	CD194 (CCR4)	BV421	1G1	5 µL	562579
	CD185 (CXCR5)	BV480	RF8B2	5 µL	566142
	CD45RA	BV605	HI100	5 µL	562886
	CD127	BV711	HIL-7R-M21	5 µL	563165
	CD196 (CCR6)	BV786	11A9	5 µL	563704
Blue 488 nm	CD25	BB515	2A3	5 µL	564467
	CD294 (CRTH2)	PE	BM16	5 µL	563665
	Live/Dead	7-AAD	N/A	5 µL	559925
	CD183 (CXCR3)	PE-Cy™7	1C6/CXCR3	5 µL	560831
Red 640 nm	CCR10	APC	1B5	0.15 µL	564771
	CD3	Alexa Fluor® 700	UCHT1	0.6 µL	557943
	CD4	APC-H7	SK3	5 µL	641398

Protocol

Fresh human PBMCs from healthy donors (N=5) resuspended in BD Pharmingen™ Stain Buffer (FBS) were stained with a pre-staining cocktail of CD194 BV421, CD185 BV480, CD196 BV786, CD294 PE and CD183 PE-Cy™7 in BD Horizon™ Brilliant Stain Buffer at 37°C for 10 minutes in the dark. In a separate tube, a staining cocktail of the remaining antibody-fluorochromes was prepared with BD Horizon™ Brilliant Stain Buffer and this cocktail was added to the pre-stained cells. The cells were incubated at room temperature for an additional 20 minutes in the dark. The stained cells were then washed twice in BD Pharmingen™ Stain Buffer (FBS), resuspended in the same buffer and 7-AAD was added before the acquisition. The indicated amount of antibody was used per test (up to $1x10^6$ cells). Samples were acquired on a 3-laser (Violet/Blue/Red), 12-fluorescent parameter BD FACSLyric™ Flow Cytometer. Optimal performance of this panel was also confirmed on a 3-laser (Violet/Blue/Red), 12-fluorescent parameter BD FACSCelesta™ Flow Cytometer. The panel can also be run on any 3-, 4- or 5-laser flow cytometer with equivalent filter configuration, although panel performance may vary between different instruments.

Figure 1

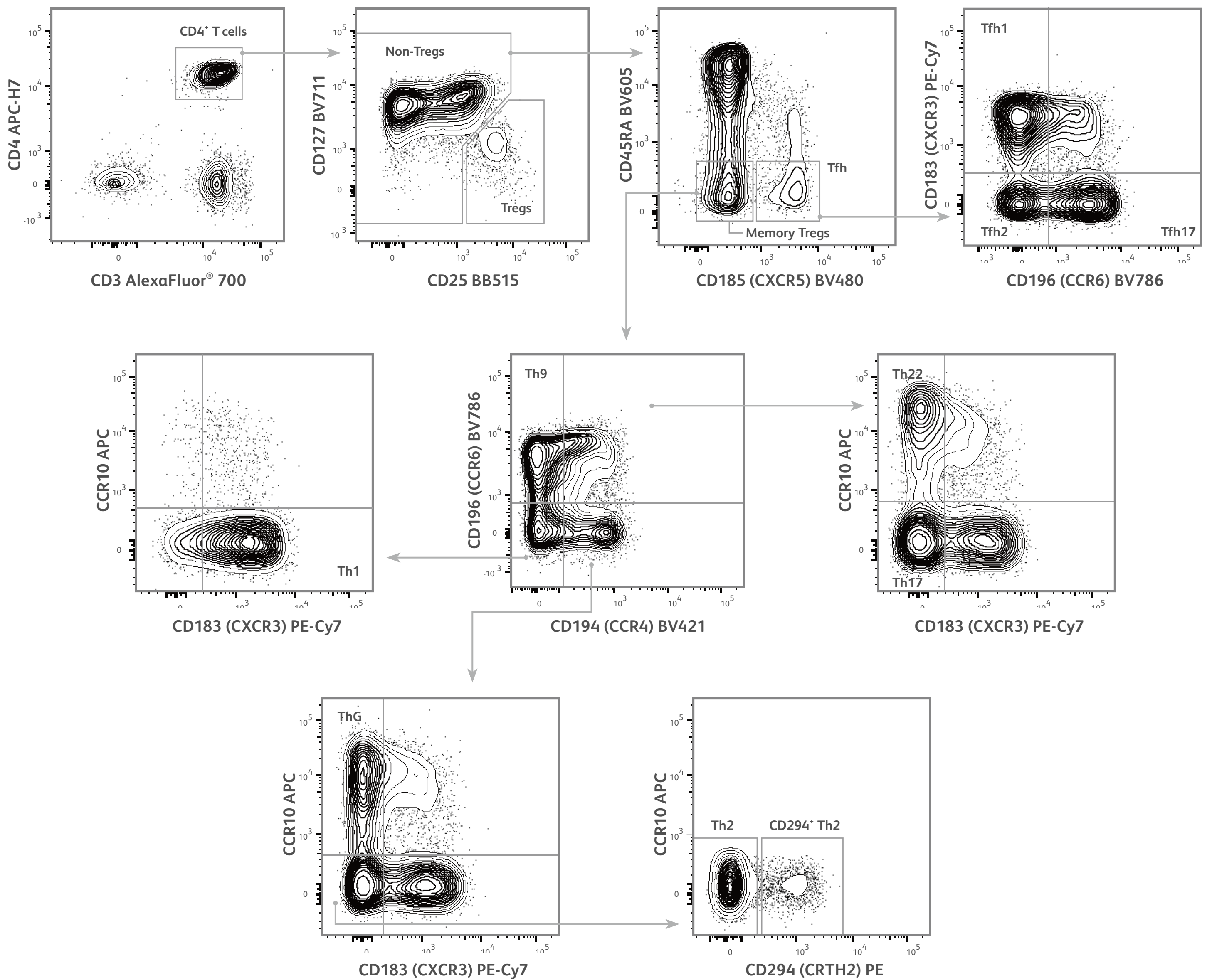

Figure 1. Identification of subsets of human circulating conventional CD4⁺ T helper cells and T follicular helper cells

T cells were first gated based on scatter properties typical of lymphocytes (not shown). After gating on CD3$^+$CD4$^+$ T cells, regulatory T cells (Tregs) and conventional T cells (non-Tregs) could be identified based on differential expression of CD127 and CD25. From the not-Tregs gate, conventional memory T cells and T follicular helper cells (Tfh) were defined as CD45RA$^-$CXCR5$^-$and CD45RA$^-$CXCR5$^+$ cells, respectively. Tfh could be further dissected into CXCR3$^+$CCR6$^-$ Tfh1, CXCR3$^-$CCR6$^-$ Tfh2 and CXCR3$^-$CCR6$^+$ Tfh17 subsets. From the memory T cells gate, Th1, Th2, Th17, Th22, Th9 and ThG subsets were defined based on differential expression of CXCR3, CCR4, CCR6, CCR10 and CD294 (CRTH2) using a gating strategy adapted from Wingender et al[3].

Figure 2

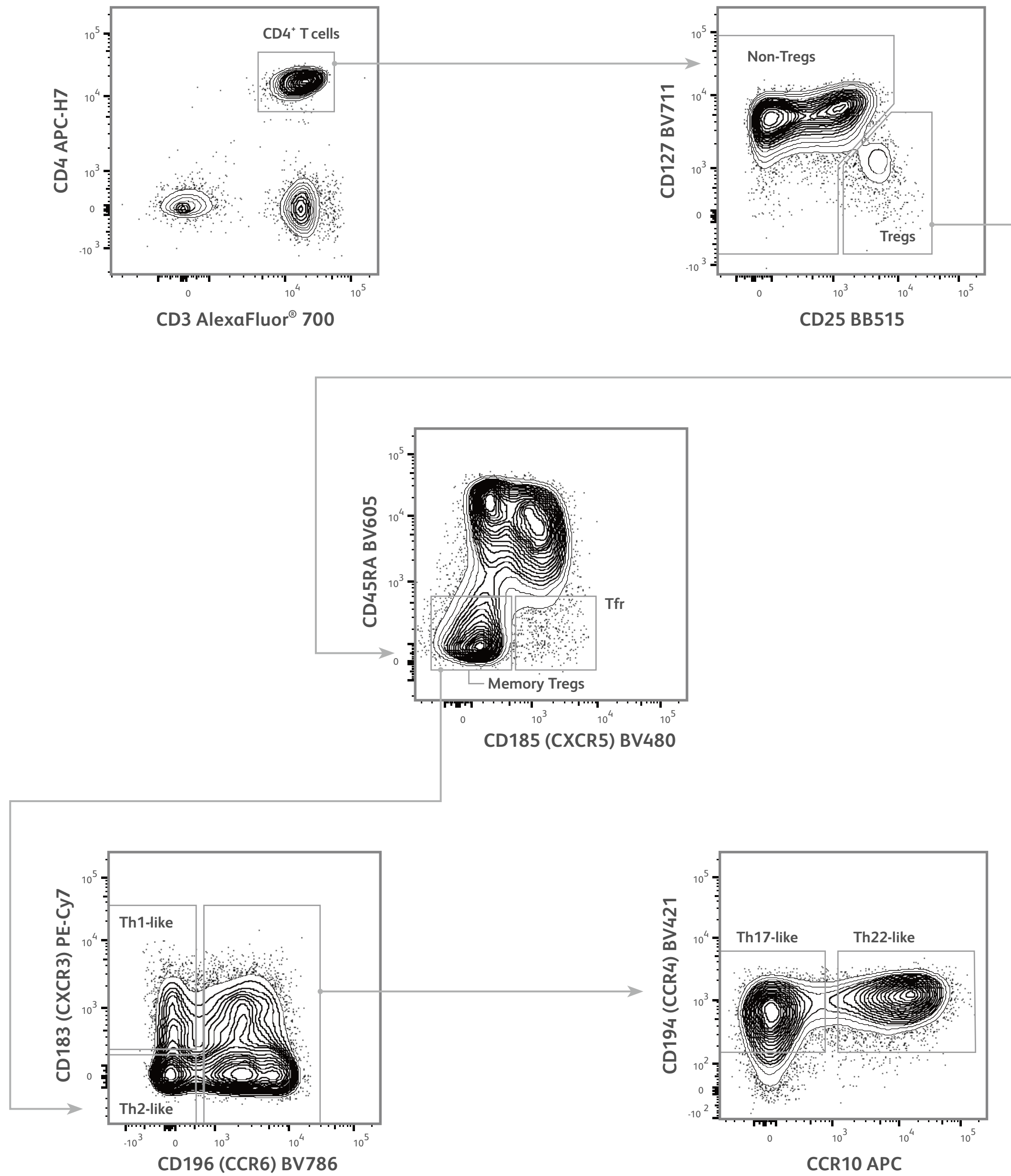

Figure 2. Identification of subsets of human circulating Tregs

T cells were first gated based on scatter properties typical of lymphocytes (not shown). After gating on $CD3^{+}CD4^{+}$ T cells, regulatory T cells (Tregs) and conventional T cells (non-Tregs) could be identified based on differential expression of CD127 and CD25. From the Tregs gate, memory Tregs and T follicular regulatory cells (Tfr) were defined as $CD45RA^{-}CXCR5^{-}$ and $CD45RA^{-}CXCR5^{+}$ cells, respectively. Memory Tregs could be further dissected into $CXCR3^{+}$ $CCR6^{-}$ Th1-like, $CXCR3^{+}CCR6^{-}$ Th2-like, $CXCR3^{+/-}CCR6^{+}CCR4^{+}CCR10^{-}$ Th17-like and $CXCR3^{+/-}$ $CCR6^{+}CCR4^{+}CCR10^{+}$ Th22-like subsets.

Conclusion

The data show the performance of the 12-color $CD4^{+}$ T cell subset panel for a clear resolution of at least 15 subsets of human circulating $CD4^{+}$ T cells.

References:

1. Morita R, et al. Human blood CXCR5+CD4+ T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity.* 2011;34(1):108-21. doi: 10.1016/j.immuni.2010.12.012.

2. Duhen T, et al. Functionally distinct subsets of human FOXP3+ Treg cells that phenotypically mirror effector Th cells. *Blood.* 2012;119(19):4430-40. doi: 10.1182/blood-2011-11-392324.

3. Wingender G, Kronenberg M. OMIP-030: Characterization of human T cell subsets via surface markers. *Cytometry A.* 2015;87(12):1067-9. doi: 10.1002/cyto.a.22788.

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