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OMIP-004: In-Depth Characterization of Human T Regulatory Cells

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Key terms

human; T cells; Treg

Purpose and Appropriate Sample Types

The present panel was constructed for the in-depth characterization of human T regulatory cells in both health and disease. The panel works well in both fresh and cryopreserved PBMCs (Table 1). The panel has also been tested on ACK-lysed peripheral blood. No other types of tissues have been tested.

Background

T regulatory cells (Tregs) are a subset of CD4 T cells, which are capable of suppressing immune responses of other T cells. In the peripheral blood of healthy individuals, these constitute approximately 3–5% of CD4 T cells. Tregs have been identified by a number of differing approaches, involving some, or all, of the following marker expressions: $CD25^{high}$, Forkhead box protein 3 (FoxP3), and $CD127^{low}$ (1–3). In the current panel, all of these markers were used, as well as additional markers such as CD39 that have been reported to be associated with high suppressive activity among Tregs (4). Antibodies to identify naïve and memory T cells, including CD45RA, CD27, and CD197 (CCR7) have been added to enable classification of Tregs in this manner (5–7). Finally, additional markers for gating (live/dead, CD45, CD3), and for cell activation (CD38, HLA-DR, CD103) were added to this panel (Supporting Information Table 2).

After testing several fluorochromes, R-phycoerythrin was reserved for FoxP3, as this marker was crucial for Treg identification. As this tube is part of a larger T cell panel with recurring markers, such as CD45, CD3, CD4 and CD8, an effort was made to assign these markers to fluorochromes that might not be widely available for other markers. Numerous clones and fluorochrome conjugates of antibodies were tested to optimize staining and minimize compensation (Supporting Information Table 3). FoxP3 is an intracellular antigen and requires the cells to be fixed and permeabilized for staining. This was performed after staining of the surface markers with the other antibodies. The use of the aqua blue live/dead stain still permitted exclusion of dead cells from analysis. Figure 1 illustrates the staining

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Additional Supporting Information may be found in the online version of this article.

achieved with this panel, and a gating strategy that can be used to identify salient populations (Supporting Information Table 6).

As Tregs are a minor component of CD4 T cells in the peripheral blood, it is necessary to acquire a large number of cells to accurately enumerate the various Treg subsets encountered. It is desirable to acquire a minimum of 50,000 CD4 T cells for analysis, and substantially more if possible. Thus, data files resulting from running this tube generally contained between 300,000 and 1,000,000 events.

Similarity to Published OMIPs

This panel can be used to measure naïve and memory CD4 and CD8 populations in a manner similar to OMIP-001.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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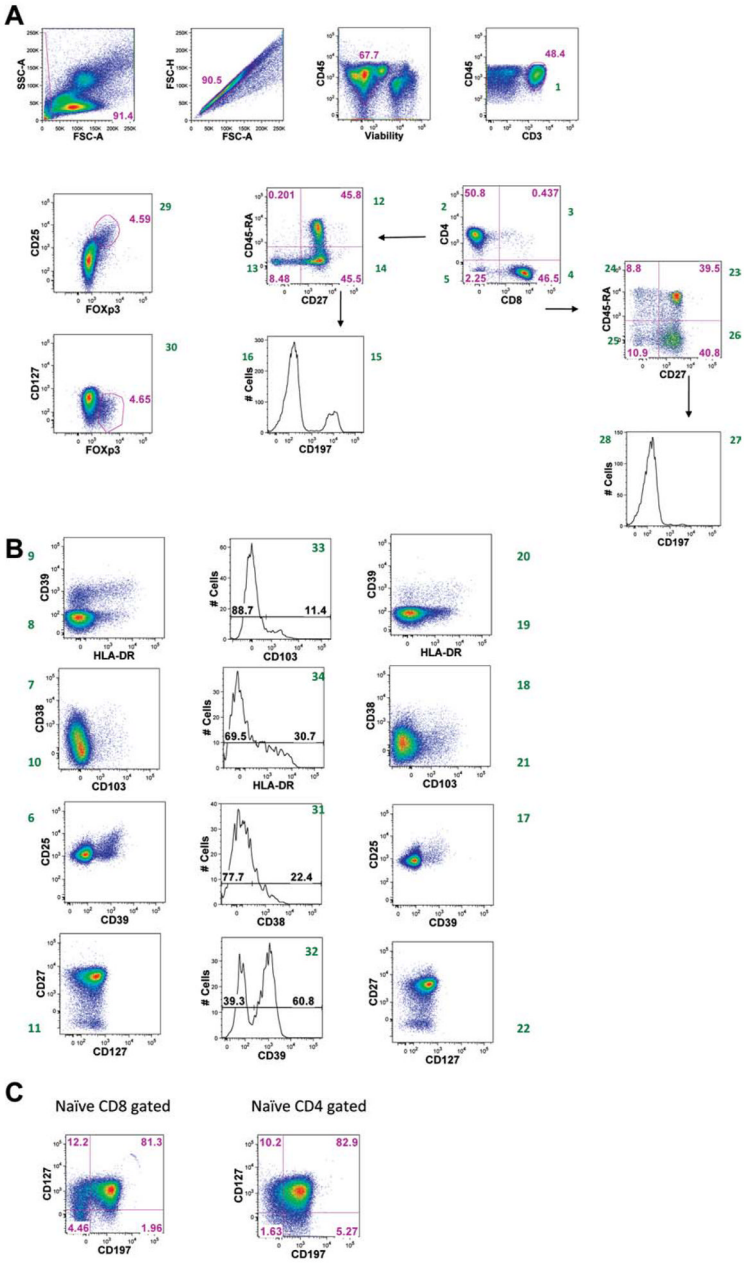

Figure 1.

An example of staining patterns and gating strategies for all fluorescent parameters. PBMC were stained according to the OMIP-004 protocol. (**A**) Debris and doublets were excluded from analysis. Live mononuclear cells were selected for sequential analysis. T cells were identified by CD3 expression. T cells subsets were identified by expression of CD4 and/or CD8. The CD4 subset was examined for expression of CD25 and Foxp3. (**B**) Representation of remaining parameters, in CD8+ T cells (left), Treg (center) and CD4+ T cells (right). (**C**) Expression of CCR7 and CD127 on naive CD8+ (left) and naive CD4+ T cells (right). Naive cells were gated as indicated in Figure 1A, through CD45RA and CD27 bivariate dot plots. Numbers in green next to the dot plots are a reference to the populations reported in online Supporting Information Table 6.

Table 1

Summary table for application of OMIP-004

Purpose	Determine Treg levels and to provide an in depth characterization of Tregs
Species	Human
Cell types	Fresh or cryopreserved PBMCs, ACK-lysed blood
Cross reference	OMIP-001

Table 2

Reagents used for OMIP-004

SPECIFICITY	FLUOROCHROME	CLONE	PURPOSE
Dead Cells	Aqua blue	na	Viability
CD45	QD800	HI30	Leukocyte gating
CD3	APC-Cy7	Sk7	T cell
CD4	V450	RPA-T4	Helper subset
CD8	QD605	3B5	Suppressor subset
CD25	PE-Cy7	M-A251	Treg gating
CD27	QD655	CLB-27/1	
CD38	PcP-Cy5.5	HIT2	
CD39	AF488	A1	Treg suppression
CD45RA	PE-TR	2H4LDH11LDB9	Naïve
CD103	PE-Cy5	LF61	
CD127	AF647	hIL-7R-M21	Treg gating
CD197	AF700	150503	
HLA-Dr	PE-Cy5.5	Tu96	
FoxP3	PE	PCH101	Treg marker