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OMIP-020: Phenotypic Characterization of Human $\gamma\delta$ T-cells by Multicolor Flow Cytometry

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• **Key terms**
$\gamma\delta$ T-cells; differentiation phenotype; human ageing; flow cytometry

Purpose and Appropriate Sample Types

This panel was composed and optimized to investigate the differentiation stages of human $\gamma\delta$ T-cells in cryopreserved peripheral blood mononuclear cells (PBMC) from healthy individuals (Tables 1 and 2). As the majority of pan-$\gamma\delta$ T-cell antibodies available commercially proved to be inappropriate for detecting all $\gamma\delta$ T-cell populations in combination with other markers, this panel provides an essential tool for the analysis of different subsets of human $\gamma\delta$ T-cells by flow cytometry. The panel works very well with cryopreserved PBMC. Other tissues have not been tested.

Background

Based on our long-standing experience of studying human $\alpha\beta$ T-cells [e.g., (1–3)], and an extensive survey of the published literature, we aimed to set up an antibody panel for the analysis of the differentiation status of circulating human $\gamma\delta$ T-cells. During the initial phase of panel development, we became aware that the majority of commercially available anti-$\gamma\delta$ TCR antibodies do not detect all $\gamma\delta$ T-cell subsets when used in combination with other markers in a multicolor flow cytometry panel. The following pan-$\gamma\delta$ T-cell antibodies were tested: an APC-conjugated antibody from clone B1, a PE-conjugated antibody from clone B1.1, and unconjugated, PE-Cy7- and FITC-conjugated antibodies from clone 11F2. Of these, the only pan-$\gamma\delta$ antibody we found able to stain all $V\delta1^+$ T-cells (as identified by antibodies from clones TS8.2 & R9.12) and all $V\delta2^+$ T-cells (as identified by antibodies from clones B6 & Immu 389), in a multicolor panel, was the unconjugated form of the clone 11F2 antibody. Having found a suitable marker for identification of all $\gamma\delta$ T-cells, other markers were added to the panel.

The panel as finally developed includes a live/dead marker^{EMA}, CD3$^{Alexa\ 700}$, CD4^{PerCP}, CD8$^{APC\text{-}H7}$, and the aforementioned pan-$\gamma\delta$ TCR$^{indirect\ PO}$, as a basis to identify $\gamma\delta$ T-cells. This combination allows the analysis of all $\gamma\delta$ T-cells and to distinguish the $CD4^-CD8^-$ cells from those expressing CD8 (4,5) (gating strategy provided in Supporting Information). Furthermore, $V\delta1$ TCR^{FITC} and $V\delta2$ TCR^{PerCP} markers were used to discriminate the two main $\gamma\delta$ T-cell populations in peripheral blood, also allowing the analysis of the remaining small pool of $V\delta1^-V\delta2^-$ circulating $\gamma\delta$ T-cells. EMA can be replaced by red dead stain without altering the performance of the panel (Supporting Information Fig. 8). It also works well after fixation

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Table 1. Summary for application of OMIP-020

PURPOSE	$\gamma\delta$ T-CELL DIFFERENTIATION PHENOTYPE
Species	Human
Cell types	PBMC
Cross references	n.a.

Table 2. Reagents used for OMIP-020

SPECIFICITY	FLUROPHORE	CLONE
CD 3	Alexa 700	UCHT-1
CD 4	PE-Cy7	OKT4
CD 8	APC-H7	SK1
CD 16	BV 711	3G8
CD 27	APC	0323
CD 28	PE	CD28.2
CD 45 RA	Pacific Blue	H100
$\gamma\delta$-TCR	Purified	11F2
Vδ1-TCR	FITC	TS8.2
Vδ2-TCR	PerCP	B6
F(ab′)2-Fragment goat anti-mouse	Pacific Orange	
Dead cells	EMA	

and permeabilization (Supporting Information Fig. 5). CD27^{APC} and CD28^{PE} in combination with CD45RA$^{Pacific\ Blue}$ was informative for the differentiation phenotype of the $\gamma\delta$ T-cell subsets as previously reported in several studies (5–14). Finally, we have included CD16^{BV711} in this panel, as it reportedly distinguishes between $\gamma\delta$ T-cell subsets with different activation pathways and functional properties (15–17) (Fig. 1).

In the process of setting up the panel, fluorescence minus one (FMO)-controls were measured for each marker to identify the best antibody and fluorophor combinations in the context of the whole panel. This panel has been used successfully for analyzing more than 250 healthy individuals (and cancer patients). Data using a slimmed-down version of this panel (without CD16) were recently published (17).

Similarity To Other Published OMIPs

Thus far, there are no other OMIPs published for $\gamma\delta$ T-cells.

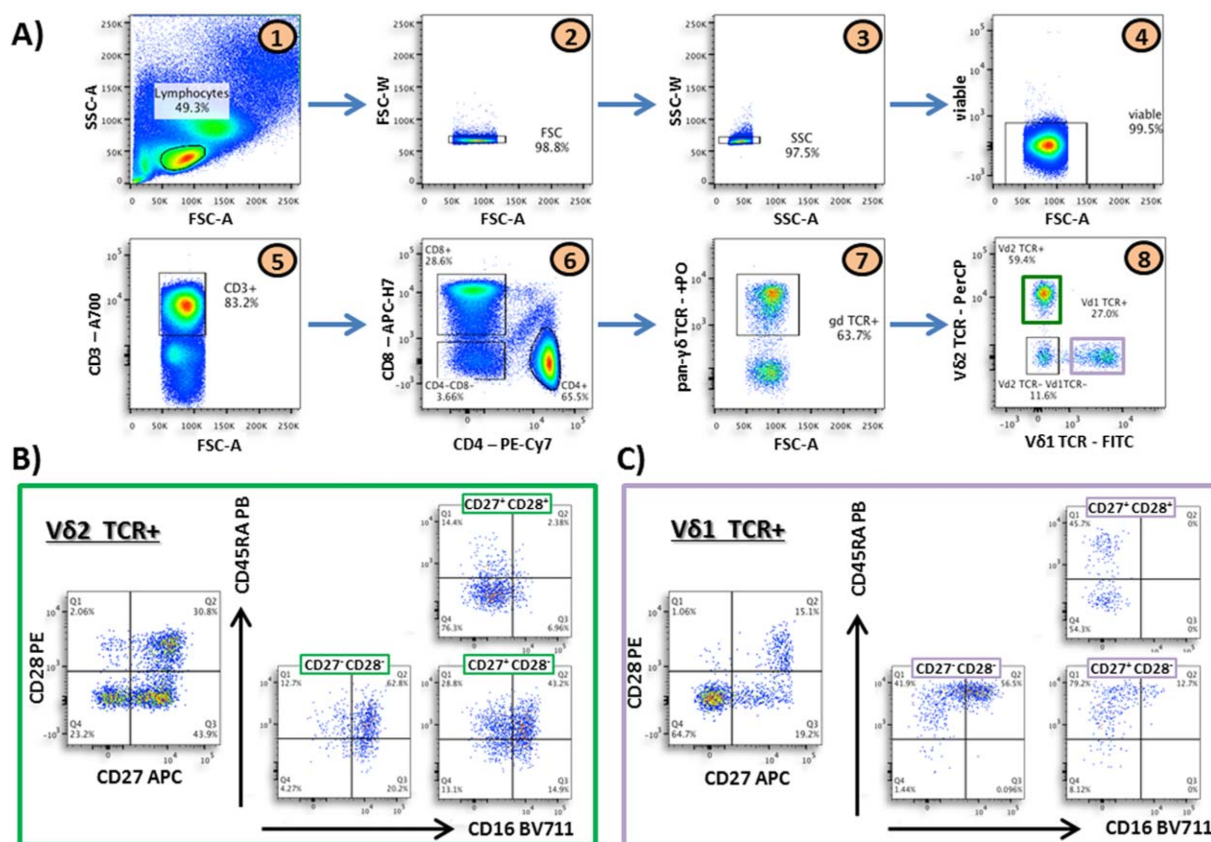

Figure 1. Example of the gating strategy. (**A**) Gating of the sample starts with a time gate (not shown) to avoid cross-contamination followed by lymphocyte gating (1) and singlet-gating (2 and 3). To exclude dead cells, an EMA- "viable gate" (4) is set, followed by gating the CD3$^+$ population (5) and the discrimination of the T cells into CD4 and CD8 subsets (6). CD4$^-$CD8$^-$ cells were then gated to enrich for $\gamma\delta$ T-cells (7) which were further discriminated into Vδ1$^+$, Vδ2$^+$, and Vδ1$^-$Vδ2$^-$ subpopulations (8). (**B**) Differentiation phenotyping of Vδ2 TCR$^+$ and Vδ1 TCR$^+$ (**C**) cells by their expression of CD27, CD28, CD45RA, and CD16. (The dot size in (B) and (C) is larger than in (A) to display the small populations better). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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