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Cytometry PART A
Journal of the International Society for Advancement of Cytometry

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OMIP-021: Simultaneous Quantification of Human Conventional and Innate-Like T-Cell Subsets

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Received 12 January 2014; Revised 1 April 2014; Accepted 6 April 2014

Grant sponsor: National Health and Medical Research Council of Australia Program Grant, Grant number: 1013667; Grant sponsor: Leukaemia Foundation of Australia Postgraduate Scholarship (number); Grant sponsor: NHMRC Senior Principal Research Fellowship, Grant number: 1020770.

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

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Published online 22 April 2014 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cyto.a.22475

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Purpose and Appropriate Samples

This panel was developed in order to simultaneously quantify both conventional peptide-MHC-restricted, and innate-like T-cell compartments in human peripheral blood samples. The panel can assess the dynamics of naïve through to terminally differentiated effector memory T-cell subsets, as well as enumerating natural killer T (NKT) cells, mucosal-associated invariant T (MAIT) cells, $\gamma\delta$ T-cells, and subsets thereof. The panel is suitable for use on both, freshly isolated or cryopreserved peripheral blood mononuclear cells (PBMC). Staining may be performed in a 96-well plate to increase throughput.

Background

Investigating T-cells based on their differentiation and activation status allows broad tracking of the conventional T-cell dynamics by longitudinal data analysis, and is widely used in clinical research. This allows researchers to define changes in T cell responses to specific insults such as infectious disease or cancer and to describe aberrations in T cell homeostasis (1).

Currently, there is growing interest in a group of T-cells collectively known as "innate-like T-cells". These include CD1d-restricted, lipid antigen-reactive, NKT cells. Two broad classes of these cells exist (2): Type-1 NKT cells are the most widely studied, and express an invariant TCR-α chain consisting of TRAV10 (Vα24) joined to TRAJ18 (Jα18), paired with a limited array of TCR-β chains that utilize the TRBV11 (Vβ11) gene with a range of TRDB (Dβ) and TRBJ (Jβ) genes. Type-1 NKT cells are also defined by their ability to recognize the prototypic glycolipid antigen, α-Galactosylceramide (αGalCer). Type-2 NKT cells are also CD1d restricted, but use a broad range of TCR-α and TCR-β chains and recognize other lipid-based antigens but not αGalCer. Another intriguing population of innate-like T cell is the MHC-related protein 1 (MR1)-restricted Mucosal-Associated Invariant T (MAIT) cells. These cells also express an invariant TCR-α chain, TRAV1-2 (Vα7.2) joined to TRAJ33 (Jα33), paired predominantly to TCR-β chains using the TRBV6 (Vβ13) and TRBV20 (Vβ2) family genes (3), and recognize Vitamin B metabolite antigens derived from microbial Riboflavin metabolism (4). And finally, $\gamma\delta$ T-cells represent an entirely distinct lineage of T cell that utilize TCR-γ and TCR-δ genes to form their CD3 associated TCR heterodimer (5). $\gamma\delta$ T cells appear to recognize a broad range of antigens, including lipid antigens, phosphoantigens as well as MHC and MHC-like molecules (6).

Innate-like T cells behave in a fundamentally different manner from conventional T-cells in that they circulate with an effector memory phenotype, poised to rapidly expand and surmount a response upon stimulation, thus acting as peripheral sentinels of the immune system. Furthermore, these cells display broad antigen reactivity to both endogenous and exogenous antigens, typically although not always, via restric-

Table 1. Summary table for application of OMIP-021

PURPOSE	NKT CELLS, $\gamma\delta$ T-CELLS, MAIT CELLS, MEMORY T-CELL SUBSETS
Species	Human
Cell Types	PBMC
Cross References	OMIP-019

tion to nonpolymorphic MHC class I-like molecules. Innate-like T-cells play roles in microbial immunity, tumor immunity, and tissue homeostasis (7–9). Importantly, the innate-like T-cells can make up to 20% of the human peripheral blood T-cell pool, although they are highly variable in frequency and number (10). In addition, fluctuations in their peripheral blood frequencies have been linked to several disease states (11–20).

In depth analysis of multiple T-cell subsets from clinical samples can be difficult because of limited sample availability and limited cell numbers per sample. For example, Type-1 NKT cells (from here on referred to NKT cells for the sake of simplicity) are relatively rare in human peripheral blood (typically between 0.01% and 0.5% of total T-cells) (21). Therefore in order to collect sufficient events for their phenotypic analysis, between 100,000 and 1,000,000 PBMCs must be analyzed.

Table 2. Reagents used for OMIP-021

SPECIFICITY	FLUOROCHROME	CLONE	PURPOSE
Dead cells	ViViD	–	Dump
CD3	BV785	OKT3	Lineage
CD4	APC-Cy7	RPA-T4	Phenotyping
CD8α	BV650	RPA-T8	
CD8β	APC	2ST8.5H7	
CD27	BV711	O323	Memory
CD28	PE-Cy5	28.2	T-cell Subsets
CD45RA	PerCP-Cy5.5	HI100	
CD45RO	AF700	UCHL1	
CCR7	PE-Cy7	3D12	
hCD1d-PBS44	BV421	n/a	NKT cells
TCR$\gamma\delta$	FITC	11F2	$\gamma\delta$ T-cells
TRAV1-2	PE	3C10	MAIT cells
CD161	BV605	HP-3G10	

Furthermore, analysis of conventional T-cells and subsets thereof can be confounded by large fluctuations in the innate-like T-cell populations, which often go unnoticed because of a lack of specific markers in monoclonal antibody (mAb)/tetramer cocktails (8).

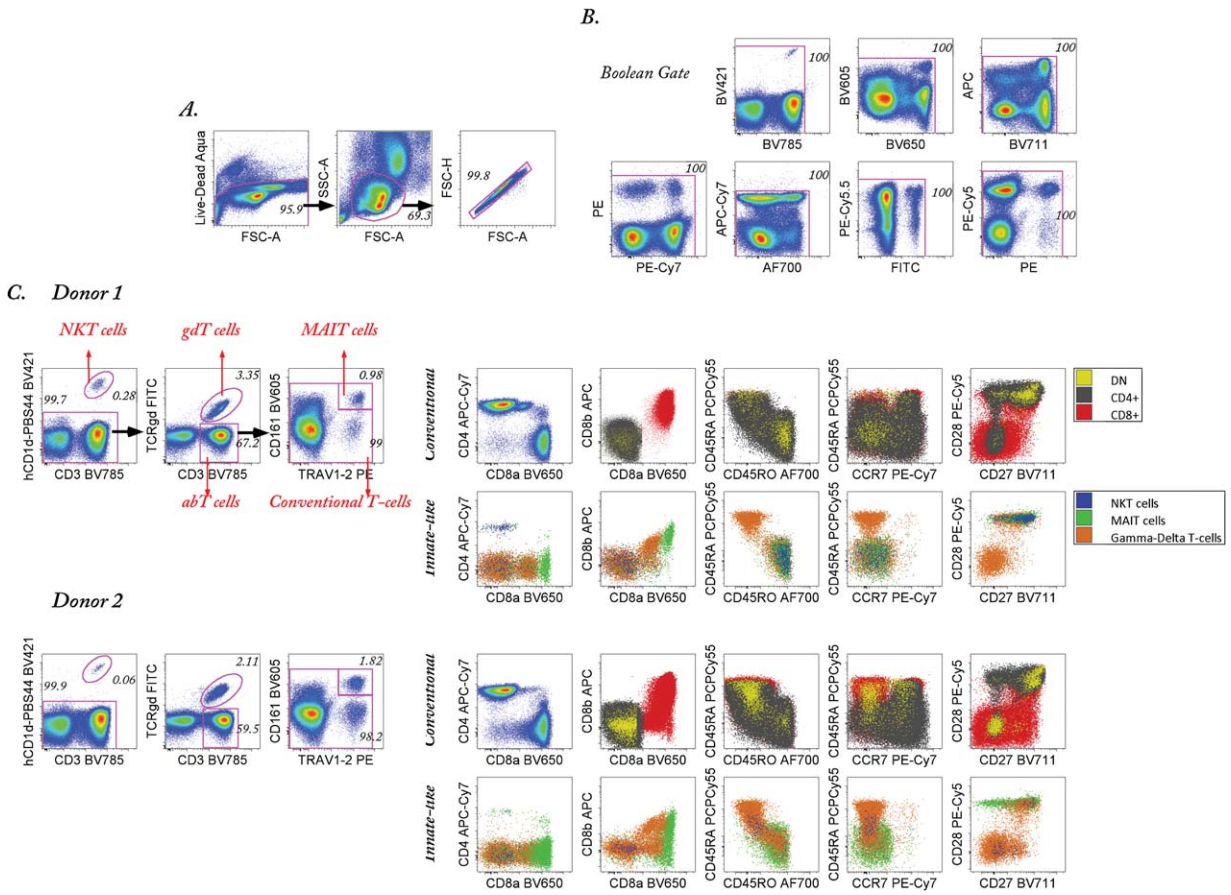

Figure 1. Multiparameter Gating strategy. (A) Lymphocyte gating strategy involves exclusion of dead cells followed by gating on the bulk lymphocyte population based on FSC-A vs SSC-A. Doublets are then removed (as shown?) and then (b) lymphoid cells are subjected to boolean gates to remove dye aggregates. (c) NKT-cells are gated using CD3 vs αGalCer-loaded CD1d tetramers. CD3 vs TCR$\gamma\delta$ is then used to discriminate between $\gamma\delta$ and $\alpha\beta$ T-cells. $\alpha\beta$ T-cells are subsequently gated for MAIT cells using TRAV1-2 vs CD161.

We therefore developed this flow cytometric panel (Tables 1–2, and Online Methods) of reagents in order to assess the conventional T-cell compartment in parallel with the innate-like T-cell subsets. We have included a mAb directed against all $\gamma\delta$ T-cell receptors (TCR) as these cells are often excluded or not separately analyzed. CD1d tetramers loaded with αGalCer specifically bind the NKT TCR (22,23), thus identifying NKT cells, and MAIT cells are identified by co-staining with a mAb directed against the TRAV1-2 (Vα7.2) variable region of the MAIT TCRα chain in combination with anti-CD161 (24). Figure 1c illustrates this gating strategy.

Most CD8+ T-cells express CD8 on their surface as a heterodimer, comprising both the CD8α and CD8β subunits. However some T-cells, including $\gamma\delta$ T-cells, $\alpha\beta$ T-cells, NKT cells, and MAIT cells, express CD8α as a homodimer (25) as depicted in Figure 1c, and these cells display innate-like characteristics. Indeed NKT cells, MAIT cells, and $\gamma\delta$ T-cells are often CD8αα+ (26). We have therefore included both CD8α and CD8β to distinguish between these sub-populations.

Finally, conventional peptide-MHC restricted T-cells generally follow adaptive-like dynamics, progressing from a naïve state through several stages of memory, each with specified function (27). For example, CD45RO/RA isoforms delineate between antigen experienced and naïve, respectively, while CCR7 and CD45RA expression allow discrimination between naïve ($CCR7^+$, $CD45RA^+$), central memory ($CCR7^+$, $CD45RA^-$), effector memory ($CCR7^-$, $CD45RA^-$) and terminally differentiated effector memory ($CCR7^-$, $CD45RA^+$) subsets as exemplified in two healthy donors in Figure 1c. CD27 and CD28 markers allow further subsetting of these cells. Importantly, distinguishing between these subsets has provided insight into several disease processes (28,29). We have thus included in our panel CD27, CD28, CD45RA, CD45RO, and CCR7 to define the differentiation status of conventional T-cells in parallel to fluctuations within the innate-like T-cell compartment (1).

Similarity to Published OMIP

OMIP-019: Quantification of Human $\gamma\delta$T-Cells, iNKT-Cells, and Hematopoietic Precursors (30).

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