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OMIP

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OMIP-019: Quantification of Human γδT-Cells, iNKT-Cells, and Hematopoietic Precursors

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Additional and updated supporting information including technical details may be found in the online version of this article.

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Purpose and Appropriate Sample Types

The present panel was optimized to quantify the relative frequencies of γδT-cells, invariant natural killer T-cells (iNKT-cells), and hematopoietic precursors in peripheral blood mononuclear cells (PBMC) from healthy individuals (Table 1). It works well with cryopreserved PBMC and we have observed similar results with fresh specimens. Other tissue types have not been tested.

Background

We developed this panel (Table 2) as part of a large study where we aim to survey the relative proportion of different immune cell subsets, including hematopoietic stem cells (HSC), in human peripheral blood specimens from healthy adults. It addresses HSC, γδT-cells, and iNKT-cells.

HSC are multipotent precursor cells that give rise to all blood cell types, including the myeloid and lymphoid lineages. Though predominantly found in bone marrow and umbilical cord blood, they also occur at reduced frequencies in the blood (1), and can be identified by their expression of CD34 (1,2). In spite of being generally used as a molecular marker of HSCs, the function of CD34 is poorly understood (3).

While most T-cells express a T-cell receptor (TCR) comprised of an α- and a β-chain, a minority of blood T-cells express the γδTCR. In healthy individuals, the vast majority of these have one of two phenotypes, representing ontologically separate lineages: $DV1^+$ (previously Vδ1) cells are prevalent during fetal and early life, while $DV2^+$ (previously Vδ2) cells usually dominate in adult blood (4,5). The latter are usually $GV9^+$ (previously Vγ9), but DV1 associates with a number of different Vγ chains (6). γδT-cells, in particular GV9/DV2 cells, are thought to act as a bridge between innate and acquired immunity (7).

iNKT-cells express the AV24/BV11 TCR (previously Vα24/Vβ11) and recognize CD1d-restricted lipid antigens. The classical antigen used to detect these cells is the marine sponge-derived α-galactosylceramide (α-GalCer), though more common environmental Ags have recently been shown to also stimulate iNKT-cells (8,9). CD1d molecules loaded with the α-GalCer analogue PBS-57 form more stable multimeric complexes than those loaded with α-GalCer, thus making a good tool to identify

Table 1. Summary table for application of OMIP-019

PURPOSE	γδT-CELLS, INKT-CELLS, HAEMATOPOIETIC PRECURSORS
Species	Human
Cell types	PBMC
Cross-references	n.a.

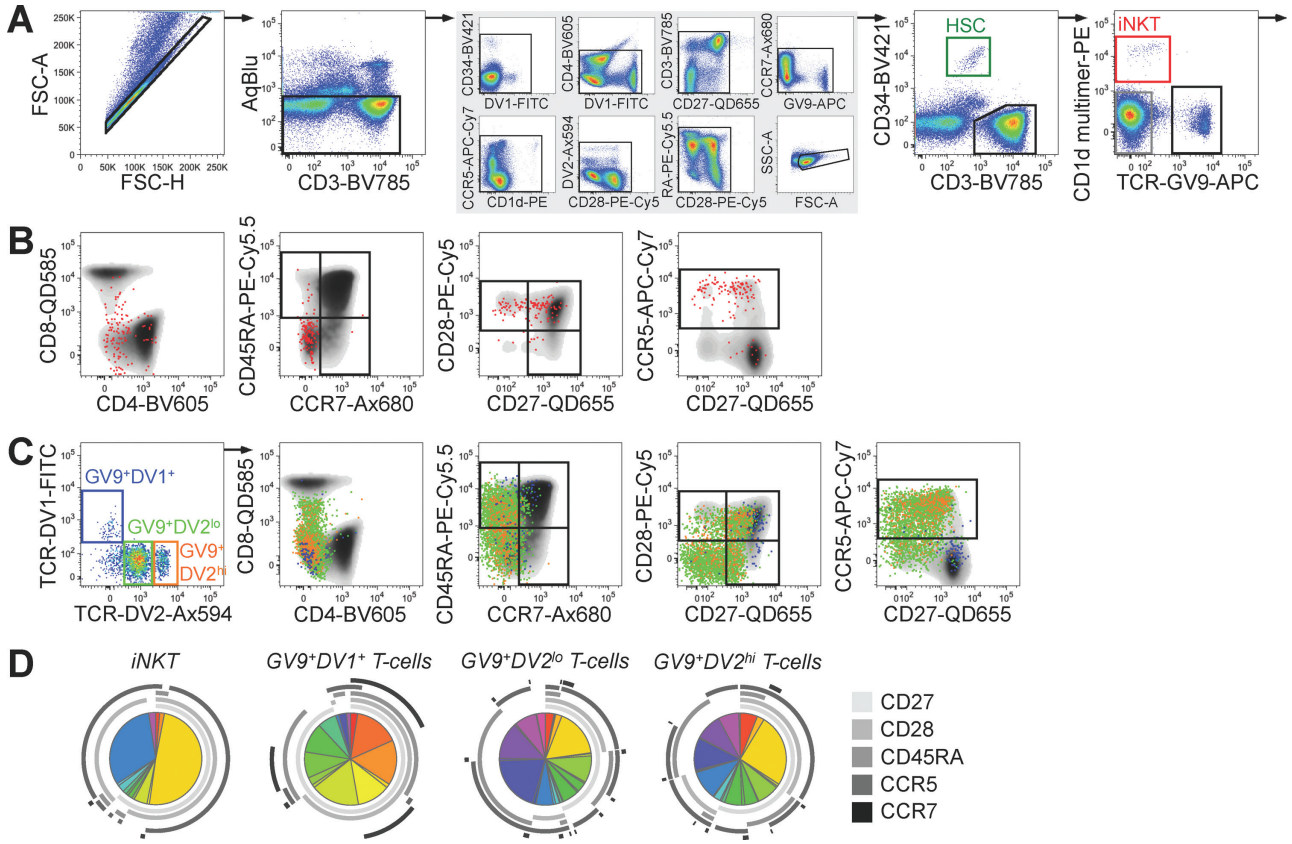

Figure 1. Example staining and gating. **A:** Identification of HSC, iNKT-cells, and TCR-GV9$^+$ $\gamma\delta$T-cells. After selecting live single lymphocytes (highly auto-fluorescent monocytes appear AqBlu^{dim} and are excluded from further analyses), eventual dye aggregates are excluded by Boolean gating (gray box) and a lymphocyte gate set. CD34$^+$ cells identify HSC (dark green gate). Within CD3$^+$ cells, CD1d-PBS57 multimer-binding iNKT-cells (red gate) and TCR-GV9$^+$ $\gamma\delta$T-cells are then selected for further analysis. Classical T-cells (gray gate) are used to define gates for remaining phenotypic markers, as shown in (B) and (C); the classical T-cells are illustrated in gray-black shades in the overlay graphs to validate gate placement. **B**: Phenotypic characterization of iNKT-cells. The expression of CD4, CD8, CD27, CD28, CD45RA, CCR5, and CCR7 is investigated on iNKT-cells (red dots) using gates defined according to the corresponding expression on classical T-cells. **C**: Identification and phenotypic characterization of $\gamma\delta$T-cell subsets. Separate GV9$^+$ T-cell subsets were identified due to differential expression of TCR-DV1 and –DV2 (blue, green, and orange dots). The expression of CD4, CD8, CD27, CD28, CD45RA, CCR5, and CCR7 is investigated using gates defined according to the corresponding expression on classical T-cells. **D**: Exploration of differentiation status of iNKT-cells and $\gamma\delta$T-cell subsets. Pie charts illustrate the co-expression pattern of CD27, CD28, CD45RA, CCR5, and CCR7 as defined by Boolean gating. While gray arcs indicate the expression of individual cell surface markers, colored pie slices identify the frequency of subsets expressing varying combinations of these markers; e.g., roughly 50% of iNKT are part of the mustard yellow slice, representing CD27$^+$CD28$^+$CD45RA$^-$CCR5$^+$CCR7$^-$ cells. For the purpose of this OMIP these pies illustrate the relative variety of phenotypes represented within iNKT and different $\gamma\delta$T-cell populations.

Table 2. Reagents used for OMIP-019

SPECIFICITY	CLONE	FLUOROCHROME	PURPOSE
CD3	OKT3	BV785	Lineage
CD1d /PBS-57 multimer	n.a.	PE	iNKT
TCR-DV1	TS8.2	FITC	$\gamma\delta$T-cells
TCR-DV2	B6	Ax594	
TCR-GV9	B3	APC	
CD34	HI100	BV421	Hematopoietic stem cells
CCR5	2D7/CCR5	APC-Cy7	Phenotyping
CCR7	150503	Ax680	
CD4	OKT4	QD605	
CD8	RPA-T8	QD585	
CD27	1A4LDG	QD655	
CD28	CD28.2	PE-Cy5	
CD45RA	MEM-56	PE-Cy5.5	
Dead cells	–	AqBlu	Dump

BV, brilliant violet; PBS-57, analogue of α-galactosylceramide; n.a., not applicable; PE, R-phycoerythrin; FITC, fluorescein; Ax, Alexa; APC, allophycocyanin; Cy, cyanine; QD, quantum dot; AqBlu, LIVE/DEAD Fixable Aqua Dead Cell Stain.

iNKT-cells (10). Three iNKT subsets have been characterized that differ in function, but also in CD4/CD8 expression: cytokine-producing $CD4^+$ $CD8^-$ (predominant in fetal and neonatal blood), cytotoxic $CD4^-$ $CD8^-$, and the rare IFN-γ-producing $CD4^-$ $CD8^+$ iNKT-cells (11).

Finally, we included Abs to CCR5, CCR7, CD27, CD28, and CD45RA in order to further explore the differentiation phenotypes of both $\gamma\delta$T-cells and iNKT-cells (Figure 1).

Similarity to Published OMIPs

None to date.

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