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Cytometry
PART A
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OMIP-002: Phenotypic Analysis of Specific Human CD8+ T-Cells Using Peptide-MHC Class I Multimers for Any of Four Epitopes

Purpose and Appropriate Sample Types

This panel was developed to determine the phenotype of human antigen (Ag)-specific CD8+ T-cells. Ag-specificities are identified by four peptide-major histocompatibility complex (MHC) class I (pMHCI) multimers (e.g., against Epstein-Barr virus (EBV) and cytomegalovirus (CMV) epitopes). Six markers of T-cell phenotype are used. This panel has been tested on fresh and cryopreserved peripheral blood mononuclear cells (PBMC), as well as bone marrow samples; staining may be performed in 96-well plates to increase throughput.

Background

Analysis of Ag-specific T-cells can be difficult because: (A) frequencies are low, (B) phenotypes are diverse, and (C) sample is typically limited. To overcome these issues, this panel assesses multiple Ag-specificities and phenotypes (1). To maximize sensitivity, some multimers are produced using quantum dots. These are bright and detected in channels receiving minimal contaminating light from other dyes. R-phycoerythrin and allophycocyanin are used for an additional multimer and a low-density Ag, respectively. Various CD27, CD127, CCR7, and CD45RO conjugates were tested; the brightest were selected. CCR7 and multimer staining were performed at 37°C (2,3).

Table 1. Summary table for OMIP-002

Purpose	Specificity and phenotype of CD8 + T-cells
Species	Human
Cell types	Fresh or cryopreserved PBMC, bone marrow mononuclear cells
Cross references	None

Various strategies are used to ensure accurate identification of Ag-specific cells. First, sources of nonspecific binding (e.g., dead cells, B-cells, and monocytes) are identified in one channel for exclusion from analysis. Second, when the same cells bind multimers against different Ag-specific cell populations, the multimers are discarded and remade. Third, preliminary experiments are repeated until nonspecific binding of the multimer to

Table 2. Reagents used in OMIP-002

SPECIFICITY	CLONE	FLUOROCHROME	PURPOSE
CD3	SK7	APC-Cy7	T-cell subset
CD4	MT-477	QD705	
CD8	RPA-T8	PE-Ax594	
Multimer 1	–	PE	Ag-specificity
Multimer 2	–	QD565	
Multimer 3	–	QD605	
Multimer 4	–	QD800	
CD45RO	UCHL1	APC-Ax700	Maturity
CCR7	150503	PE-Ax750	
CD27	1A4	PE-Cy5	
CD127	R34.34	PE-Ax700	
PD1	MIH4	Bi	
Bi	SAV	APC	
CD57	NK-1	FITC	
CD14	M5E2	PacBlu	Exclusion
CD19	HIB19	PacBlu	
Dead cells	–	ViViD	

APC, allophycocyanin; Cy, cyanin; QD, quantum dot; PE, R-phycoerythrin; Ax, alexa; Bi, biotin; SAV, streptavidin; FITC, fluorescein isothiocyanate; PacBlu, pacific blue; ViViD, LIVE/DEAD fixable violet dead cell stain.

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

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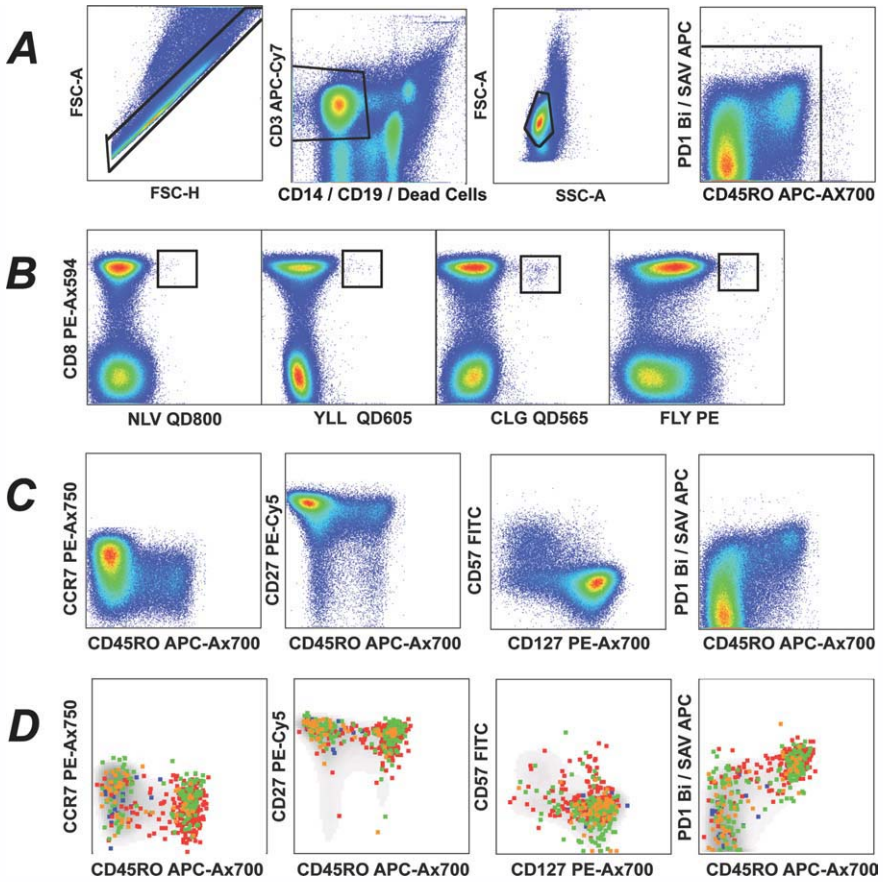

Figure 1. Staining patterns in PBMC. **A**: Overall gating strategy. Successive gates are applied to identify single cells, live CD3+ T-cells, and lymphocytes. Gates are also applied to all fluorochromes to remove fluorochrome aggregates (e.g., as shown for PD1 and CD45RO). Subsequent panels are gated on these cells. **B**: Identification of Ag-specific CD8+ T-cells. Multimer staining is depicted within CD3+ T-cells for various HLA-A2 epitopes of CMV and EBV (see Supporting Information). **C**: Phenotyping markers in bulk CD8+ T-cells. **D**: Phenotype of multimer+ CD8+ T-cells (NLV, red; YLL, blue; CLG, green; and FLY, orange). Although CCR7 expression is dim, multimer+ CCR7+ cells can be roughly distinguished from CCR7− cells. By measuring CD27, identification of central memory multimer+ cells is refined.

CD4+ T-cells is no longer observed, thus ensuring the quality of the investigator's staining technique (3).

Similarity to Published OMIPs

None to date.

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