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Cytometry PART A
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OMIP-001: Quality and Phenotype of Ag-Responsive Human T-Cells

Purpose and Appropriate Sample Types

The present panel was optimized for the evaluation of $CD4^+$ and $CD8^+$ T-cell responses to various HIV-1–derived peptide pools in peripheral blood mononuclear cells (PBMC) from HIV-1^+ individuals with differences in clinical progression. It works well with cryopreserved PBMC, and we have observed similar results with fresh specimens. Other tissue types have not been tested.

Background

The approach used for the development of this panel has been described in detail (1). Briefly, a large number of Ab-conjugates were screened for each antigen of interest, as available, to select those Ab-conjugates providing best detection. As the focus of the panel was the detection of cytokine-producing T-cells, the brightest fluorochromes were used for interleukin-2 (IL-2), interferon (IFN)-γ, and tumor necrosis factor (TNF)-α. Next, priority was given to PD-1 and CCR7, as these antigens are expressed at low molecular densities. After selecting a dump channel to exclude dead cells, B-cells and monocytes/macrophages from the analysis, a range of Ab-conjugates for other markers used for T-cell subset definition and determination of activation status were tested in the free detectors until optimal detection of all antigens was achieved. To this end, CD4-QD655, which was included in early panels, was

Table 1. Summary table for application of OMIP-001

Purpose	T-cell cytokine production after in vitro stimulation and phenotyping of cytokine-producing T-cells
Species	Human
Cell types	PBMC
Cross references	n.a.

Table 2. Reagents used for OMIP-001

SPECIFICITY	CLONE	FLUOROCHROME	PURPOSE
IFN-γ	B27	APC	Function
IL-2	MQ1-17H12	Ax488	
TNF-α	MAb11	Ax594	
CD3	SK7	APC-Cy7	Lineage
CD4	M-T477	QD605	
CD8	RPA-T8	QD585	
CCR7	150503	Ax680	Memory/ differentiation
CD27	M-T271	PE-Cy7	
CD28	CD28.2	PE-Cy5	
CD45RO	UCHL1	QD545	
CD57	NK-1	QD705	
CD127	R34.34	PE	
PD-1	MIH4	Bi	
Biotin	Streptavidin	QD655	
CD14	M5E2	PacBlu	Dump
CD19	HIB19	PacBlu	
Dead cells	–	ViViD	

APC, allophycocyanin; Ax, Alexa; Cy, cyanine; QD, quantum dot; PE, R-phycoerythrin; Bi, biotin; 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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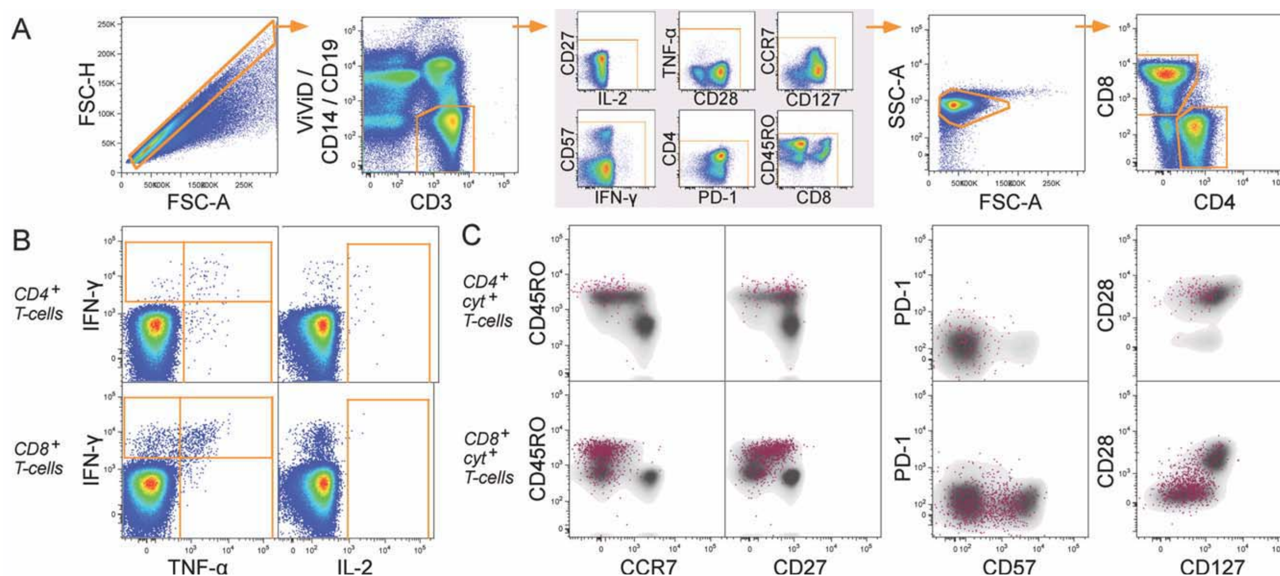

Figure 1. Example staining and gating. **A**: Identification of T-cell subsets. After selecting live $CD3^{+}CD14^{-}CD19^{-}$ single cells, eventual dye aggregates are excluded (gray box) and a lymphocyte gate set. $CD4^{+}$ and $CD8^{+}$ T-cells are then selected for further analysis. **B**: Selection of cytokine-producing cells after gating as shown in (A). $CD4^{+}$ and $CD8^{+}$ T-cells positive for either IFN-γ, TNF-α, or IL-2 are separately gated. Besides analyzing the cytokine pattern (combination of cytokines produced on a per cell basis) produced in response to antigenic stimulation, a Boolean gate encompassing all cytokine positive cells (cyt^{+}) is created to evaluate the total Ag-specific response. **C**: Phenotypic analysis of Ag-specific cells gated as described in (B). Total $CD4^{+}$ and $CD8^{+}$ T-cells (in gray) are used as a reference when analyzing the cell surface phenotype of cyt^{+} cells (in red). Shown are cryopreserved PBMC from an HIV-1^{+} subject stimulated with an HIV Gag peptide pool.

replaced with CD4-QD605 to improve the detection threshold of CD28-PE-Cy5 on $CD4^{+}$ T-cells. PD-1, which is labeled with QD655 in the final panel, does not influence the detection threshold of PD-1^{+} $CD28^{+}$ cells in the same way. This is because PD-1 has a lower expression level (and thus a lower measured mean fluorescence intensity) than CD4, thereby causing less spillover into other detectors.

CCR7 was labeled at 37°C (2), while CD3 was labeled after fixing and permeabilizing the cells, so as not to inadvertently exclude any relevant cells that might have internalized their T-cell receptor/CD3 complexes after activation (3). Fluorescently conjugated CD28 Ab was added to the stimulation cultures, thus serving as a costimulator during the cultures while at the same time, labeling CD28 molecules.

The total number of cells acquired determines the detection sensitivity of cytokine-producing cells. Thus, to reliably quantify cytokine responses, higher number of cells should be acquired as the frequency of responding cells decreases.

Similarity to Published OMIPs

None to date.

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