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OMIP-005: Quality and Phenotype of Antigen-Responsive Rhesus Macaque T Cells

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Keywords

intracellular cytokine; T cells; vaccines; HIV

Purpose and Appropriate Sample Types

The primary eight-color panel was designed to measure IFNγ, IL-2, and TNF production from viable CD4 and CD8 T cells from rhesus macaques in preclinical vaccine studies. An 11-color variant also allows for the assessment of memory subsets based on surface expression of CD28, CD45RA, and CCR7. The panel was optimized not only for use on cryopreserved peripheral blood mononuclear cell (PBMC) samples but also works well on fresh PBMC samples, cryopreserved tissue samples, and fresh tissue samples that have been treated with RBC lysis buffer (Table 1). The eight-color panel and associated staining procedure were tested in a formal qualification study and shown to be highly reproducible with low interaliquot, interday, and interanalyst variability according to the qualification criteria (manuscript in preparation).

Background

Measurement of Ag-specific T cell responses from vaccinated animals can be difficult due to low frequencies of cytokine-producing cells and high background. The approach that was used to design these panels was to prioritize the cytokine detection reagents on the brightest fluorochromes to allow for the most sensitive detection of weak responses. Then, the remaining antibody/conjugate combinations were tested to optimize detection on the other detectors (1). Because activated T cells often internalize CD3 following stimulation (2), we included anti-CD3 in the intracellular staining portion of the assay to ensure that cytokine producing cells were not excluded. To reduce background arising from cells nonspecifically binding cytokine antibodies, anti-CD69 and a live/dead discriminator were included (3,4). The eight-color panel is sufficient to measure cytokine production from CD4 and CD8 T cells, whereas the 11-color variant allows for the further discrimination of memory subsets

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(Table 2 and Figure 1). Unlike other similar panels, the anti-CD28 and anti-CCR7 antibodies were included with the other surface antibodies in a one-step stain because costimulatory antibodies were not used in this protocol and CCR7 staining at room temperature gave good separation.

Similarity to Published OMIPs

None

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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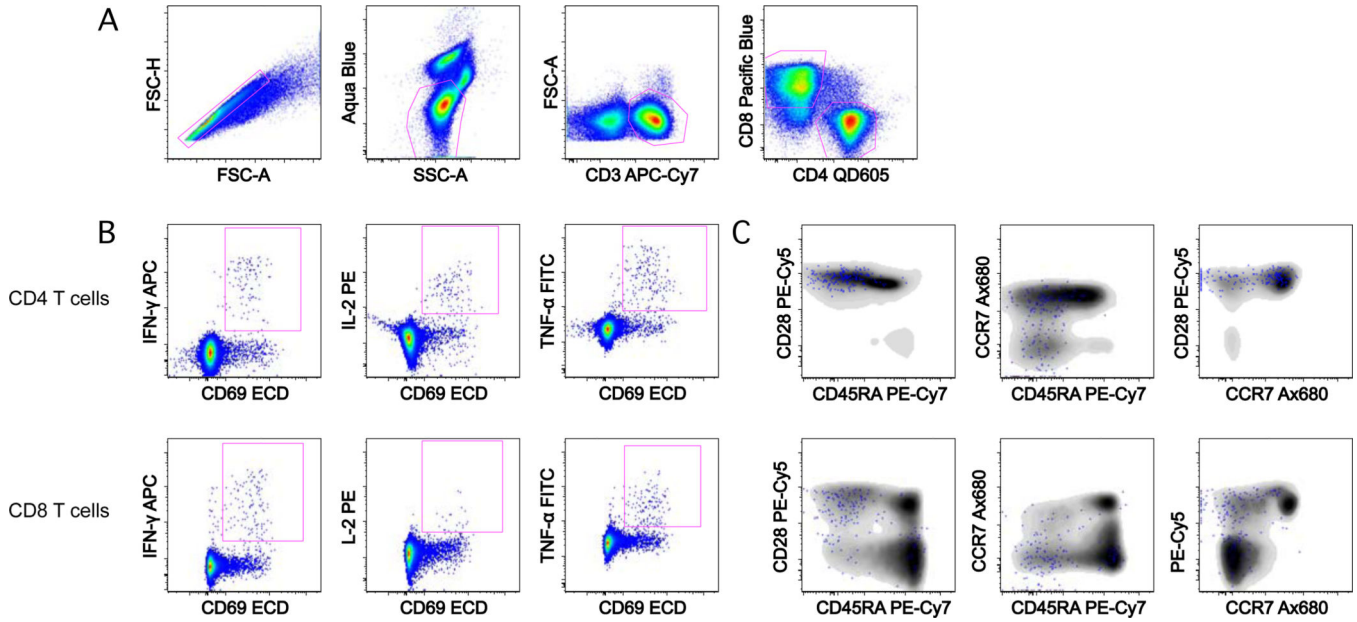

Figure 1.

Example staining and gating on rhesus macaque PBMC. (**A**) T cell subsets are selected by successive gating: first single cells, second live, SSC low cells, third CD3+, FSC low cells, and finally CD8+ and CD4+ T cells. (**B**) For each T cell subset, CD69+cytokine+ gates are drawn. (**C**) Memory subsets can be visualized by overlaying the cytokine-positive cells (IFNγ^+ or IL-2$^+$ or TNF$^+$ Boolean gate in FlowJo; blue) on the total CD4 or CD8 T cell population (gray).

Table 1.

Summary table for application of OMIP-005

Purpose	T cell cytokine production and memory phenotype
Species	Rhesus macaque
Cell types	PBMC
Cross references	(Lamoreaux et al. OMIP-009)

Table 2.

Reagents used

SPECIFICITY	CLONE	FLUOROCHROME	PURPOSE
Dead cells		Aqua Blue	Exclusion
CD3	SP34.2	APC-Cy7	T cells
CD4	S3.5	QD605	
CD8	RPA-T8	Pacific Blue	
CD69	TP1.55.3	ECD	Background reduction
IFNγ	B27	APC	Cytokines
IL-2	MQ1–17H12	PE	
TNF	Mab11	FITC	
CD45RA	L48	PE-Cy7	Memory markers (optional)
CD28	28.2	PE-Cy5	
CCR7	150503	Ax680	