Innovative, Intuitive, Flexible.

Luminex Flow Cytometry Solutions with **Guava®** and **Amnis®** **Systems**

Learn More >

Luminex®

complexity **simplified.**

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

MIFlowCyt

OMIP-017: Human CD4$^+$ Helper T-cell Subsets Including Follicular Helper Cells

Yolanda D. Mahnke,* Margaret H. Beddall, Mario Roederer

ImmunoTechnology Section, Vaccine Research Center, NIAID, NIH, Bethesda, Maryland

Received 3 December 2012; Accepted 1 February 2013

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

*Correspondence to: Y. D. Mahnke, ImmunoTechnology Section, Vaccine Research Center, NIAID, NIH, 40 Convent Drive, Bethesda, MD 20892, USA

Email: mahnkey@mail.nih.gov

Published online 28 February 2013 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cyto.a.22269

Purpose and Appropriate Sample Types

This panel was optimized to measure the relative frequencies of CD4$^+$ T-helper cell subsets and follicular helper T-cells in peripheral blood mononuclear cells (PBMC) from healthy individuals (Table 1). It works well with cryopreserved PBMC and we have observed similar result with fresh specimens. Other tissue types have not been tested.

Background

Activated CD4$^+$ T-cells differentiate into lineages, commonly referred to as T-helper (Th) subtypes such as Th_2, with unique phenotypes, cytokine signatures, and functions. The present panel (Table 2) was designed to identify major Th subsets described to date according to disparate chemokine receptor expression patterns. Immunity to intracellular infections and viruses requires CCR4$^-$ CCR6$^-$ CCR10$^-$ CXCR3$^+$ Th_1 cells that produce interferon-γ (IFN-γ) (1,2), while CCR4$^+$ CCR6$^-$ CXCR3$^-$ Th_2 cells produce interleukin-4 (IL-4), IL-5, and IL-13 and mediate the host defense against helminthes (3). Th_9 cells are CCR4$^-$ CCR6$^+$ IL-9-producing cells that could be involved in wound healing of pleural mesothelial cells during *Mycobacterium tuberculosis* infection (4). CCR4$^+$ CCR6$^+$ CCR10$^-$ CXCR3$^-$ Th_{17} cells are crucial for the host defense against extracellular pathogens, and mainly produce IL-17A, IL-22, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (2,5). Finally, CCR4$^+$ CCR6$^+$ CCR10$^+$ Th_{22} cells produce IL-22, IL-26, and IL-13 and are thought to be involved in skin immunity (2,6). However, there is plasticity in the system and some of these phenotypes and functional characteristics, previously thought mutually exclusive, can be expressed by the same cell. One such example is $Th_{17}Th_1$ cells that are CCR4$^-$ CCR6$^+$ CXCR3$^+$ and capable of producing both IFN-γ and IL-17 (5).

The affinity maturation of plasma cells in the germinal centers of B-cell follicles requires the help from another specialized CD4$^+$ T-cell lineage called follicular helper cells, or T_{FH} (7). These cells express CXCR5, which confers homing to follicular dendritic cell networks within the germinal centers.

The gating scheme for evaluating all these subsets with the present panel is illustrated in Figure 1. First, live CD4$^+$ T-cells are identified (Fig. 1A), before gating on T_{FH} (Fig. 1B) or other Th lineage cells (Fig. 1C). Additional non-lineage defining

Table 1. Summary table for application of OMIP-017

Purpose	CD4+ helper T-cell subsets (Th1, Th2, Th9, Th17, Th17Th1, Th22 and TFH)
Species	Human
Cell types	PBMC
Cross-references	n.a.

ISAC
International Society for Advancement of Cytometry

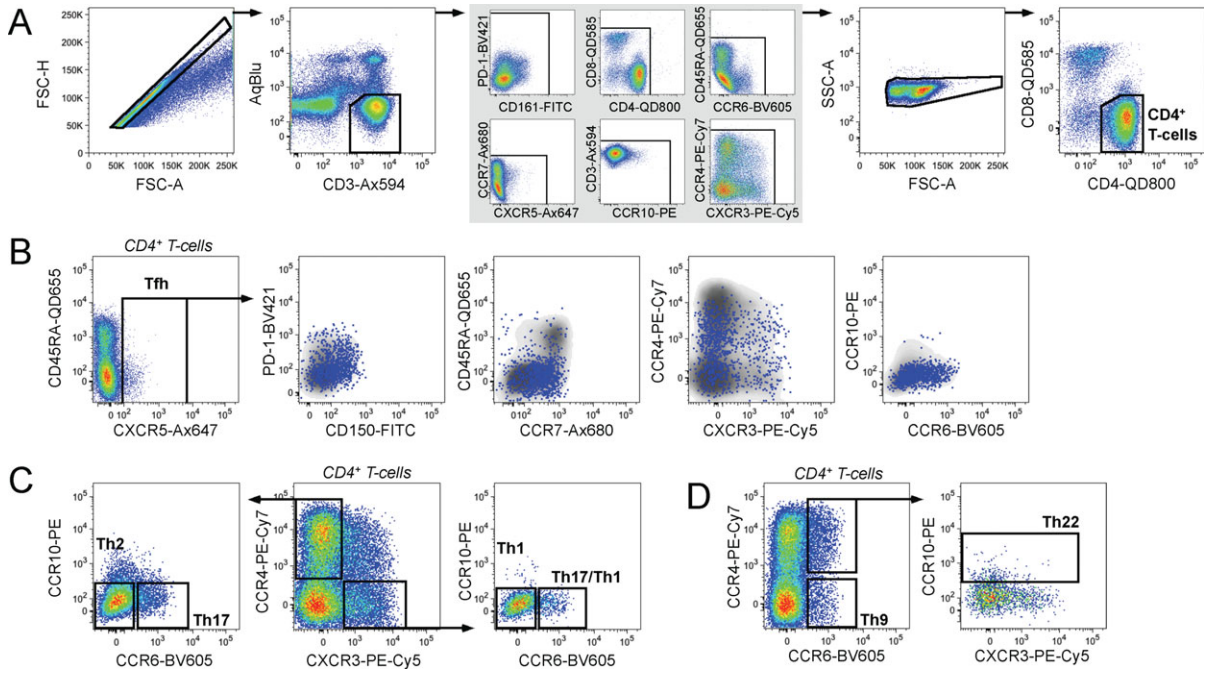

Figure 1. Example staining and gating. **A**: Identification of CD4$^+$ T-cell. After selecting live CD3$^+$ single cells, eventual dye aggregates are excluded (gray box), followed by identification of lymphocytes and CD4$^+$CD8$^-$ cells for further analysis as shown in (B, C, D). **B**: Identification of CXCR5$^+$ T_{FH} within CD4$^+$ T-cells. The phenotype of T_{FH} is investigated by overlaying these cells (blue) onto total CD4$^+$ T-cells (gray). **C**,**D**: Enumeration of CD4$^+$ helper T-cell subsets according to differential expression of the chemokine receptors CCR4, CCR6, CCR10, and CXCR3; gates indicate putative subtypes as defined in the literature (see discussion).

Table 2. Reagents used for OMIP-017

SPECIFICITY	CLONE	FLUOROCHROME	PURPOSE
CD3	UCHT1	Ax594	Lineage
CD4	OKT4	QD800	
CD8	RPA-T8	QD585	
CXCR5	RF8B2	Ax647	T_{FH}
CCR4	TG6/CCR4	PE-Cy7	Th subsets
CCR6	G034E3	BV605	
CCR10	6588-5	PE	
CXCR3	1C6/CXCR3	PE-Cy5	
CCR7	150503	Ax680	Differentiation
CD45RA	5H9	QD655	
CD161	DX12	FITC	Exploratory
PD-1	EH12.2H7	BV421	
Dead cells	–	AqBlu	Dump

Ax, Alexa; QD, quantum dot; PE, R-phycoerythrin; Cy, cyanine; BV, brilliant violet; FITC, fluorescein; AqBlu, LIVE/DEAD Fixable Aqua Dead Cell Stain.

markers, namely CCR7, CD45RA, CD161, and PD-1, were included in order to further characterize the phenotype of individual subsets.

Similarity to Published OMIPs

None to date.

Literature Cited

1. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 1998;187:129–134.
2. Duhen T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. Nat Immunol 2009;10:857–863.
3. Rivino L, Messi M, Jarrossay D, Lanzavecchia A, Sallusto F, Geginat J. Chemokine receptor expression identifies Pre-T helper (Th)1, Pre-Th2, and nonpolarized cells among human CD4+ central memory T cells. J Exp Med 2004;200:725–735.
4. Ye ZJ, Yuan ML, Zhou Q, Du RH, Yang WB, Xiong XZ, Zhang JC, Wu C, Qin SM, Shi HZ. Differentiation and recruitment of Th9 cells stimulated by pleural mesothelial cells in human *Mycobacterium tuberculosis* infection. PloS One 2012;7:e31710.
5. Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, Sallusto F, Napolitani G. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat Immunol 2007;8:639–646.
6. Zhang N, Pan HF, Ye DQ. Th22 in inflammatory and autoimmune disease: Prospects for therapeutic intervention. Mol Cell Biochem 2011;353:41–46.
7. King C, Sprent J. Emerging cellular networks for regulation of T follicular helper cells. Trends Immunol 2012;33:59–65.