HHS Public Access

Author manuscript

Cytometry A. Author manuscript; available in PMC 2019 February 01.

Published in final edited form as:

Cytometry A. 2018 February ; 93(2): 186–189. doi:10.1002/cyto.a.23303.

OMIP-042: 21-color flow cytometry to comprehensively immunophenotype major lymphocyte and myeloid subsets in human peripheral blood

Karl W Staser[1,2], **William Eades**[3], **Jaebok Choi**[2], **Darja Karpova**[2], and **John F. DiPersio**[2,*]

[1]Division of Dermatology, Department of Medicine, Washington University in St. Louis, Missouri

[2]Division of Oncology, Department of Medicine, Washington University in St. Louis, Missouri

[3]Siteman Cancer Center Flow Cytometry Facility, Washington University in St. Louis, Missouri

Keywords

T cells; B cells; myeloid cells; NK cells; dendritic cells; chemokine receptors; human blood immunophenotyping

Purpose and appropriate sample types

This 21-color flow cytometry-based OMIP[1] enables simultaneous quantification of monocytes, basophils, granulocytes, dendritic cells, natural killer cells, B cells, and all well-defined T and T helper cell subsets in the human peripheral blood. This panel captures the major phenotypes described in the NIH Human Immunology Project [2, 3] with additional markers for deep T cell analysis [4]. We specifically designed this panel for analysis of peripheral blood from patients involved in our clinical trials of novel agents for the treatment of graft versus host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (alloHSCT). We have optimized this panel for the analysis of 1×10^6 fresh or previously frozen peripheral blood mononuclear cells (PBMCs).

Background

We initially designed this panel for the analysis of the PBMCs from patients who have undergone alloHSCT, particularly those enrolled in drug studies for the prevention and treatment of GVHD. Prior studies in humans and animal models have implicated many immune cell types in the initiation and progression of GVHD, and the data have, at times, conflicted, depending on species, model, and individual laboratories. In particular, prior studies have identified imbalances in T regulatory cells (Tregs), T follicular helper (Tfh) cells, T helper type 1 (Th1), type 2 (Th2), type 17 (Th17), myeloid derived suppressor cells (MDSCs), natural killer cells (NKs), dendritic cells (DCs), and others in modulating

*Corresponding Author: John F. DiPersio, MD, PhD, Virginia E and Samuel J. Golman Professor, Chief, Division of Oncology, Deputy Director, Siteman Cancer Center, Washington University School of Medicine, 660 S. Euclid Ave, Saint Louis, MO 63110, jdipersi@wustl.edu.

The authors have no conflict of interest to declare.

engraftment, GVHD, and treatment responses [5, 6]. Accordingly, we aimed to develop a standardized panel to capture all major human lymphoid and myeloid populations with deep T cell phenotyping in a single analysis, thus reducing experimental variability, redundancy, and the need for a high quantity of input cells. As to the last point, post-HSCT patients typically have few circulating leukocytes until hematopoietic engraftment and reconstitution. Thus, multiple flow cytometry panels and/or CyTOF analyses pose a greater challenge than a single, comprehensive flow-based panel. Beyond our HSCT-focused studies, this panel should find broad application in the study of many inflammatory and neoplastic conditions. Of note, this panel uses antibodies targeting exclusively surface receptors, making fixation and permeabilization unnecessary.

After gating on FSC/SSC, single, live cells (**Figure 1A**), PBMCs broadly segregate into T cells (CD3+CD20-), B cells (CD3-CD20+), and non-B/T cells (CD3-CD20-), the latter of which includes dendritic cells, natural killer cells, myeloid, and progenitor populations (**Figure 1B**; full gating strategy **Online Table 3**). To further define non-lymphoid phenotypes described in the Human Immunology Project, we included CD14, CD16, HLADR, CD56, CD123, and CD11c surface markers. First, CD14 and HLADR distinguish monocytes (CD14+HLADR+/−) and dendritic cells (CD14-HLADR+) from other granulocytes and NKs (CD14-HLADR-) (**Figure 1C, Non-B/T**). Within this latter NK/granulocyte population, CD123 expression denotes basophils and CD56 identifies natural killer cells (**Figure 1C, NK/Granulocytes**). NK cells further segregate into at least three populations according to CD56 and CD16 density (**Figure 1C, NKs**) [7]. Within the CD16-HLADR+ DC population, CD11c and CD123 distinguish plasmacytoid DCs and monocytic DCs (**Figure 1C, DCs**). Finally, within the CD14+ monocyte population, CD16 and HLADR identify at least three populations: classical monocytes (HLADR+CD16-), non-classical monocytes (HLADR+CD16+), and a subset containing myeloid derived suppressor cells (MDSCs; HLADR-CD16-) (**Figure 1C, Monocytes**). Of note, further analyses of chemokine receptor expression can be performed on any non-B/T subset, which may have particular relevance in diseased states (data not shown).

Basic T cell markers include CD4 and CD8 (**Figure 1D, T cells**). Next, a combination of cell surface markers, including multiple chemokine receptors, identifies T cell activation, T regulatory cells (Tregs), T cell memory status, and all major Th subsets [3, 4, 8, 9]. Of note, HLADR and CD38 expression identifies T cell activation status within any subset [10], with an example shown for all CD4+ cells. Within the CD4+ T cell population, Tregs identify as CD25+CD127-/lo, a population highly correlated with Tregs traditionally defined as FOXP3+ CD4+ [8, 11, 12]. CD45RA and CCR7 further define CD4 and CD8 T cells into four major subsets: T effector cells (Teff; CD45RA+CCR7-), naïve T cells (Tnaive; CD45RA+CCR7+), T effector memory cells (Tem; CD45RA-CCR7-), and T central memory cells (Tcm; CD45RA-CCR7+) (**Figure 1D,** second panel and **Figure 1E**, first panel). Within the CD4+ T memory population (i.e. all cells that are CD20-CD3+CD4+CD45RA-), various chemokine receptors distinguish Th1, Th2, Th9, Th22, a subset containing T follicular helper cells (Tfh), and T GM-CSF-secreting (ThGM-CSF) cells [4]. First, within the T memory population, CCR10 and CXCR5 expression identify the subset containing Tfh cells (CCR10-CXCR5+) (**Figure 1D, Tem and Tcm CD4 cells**).

Within the CCR10-CXCR5-Th subset, Th9 cells can be identified as CCR6+CCR4-(**Figure 1D, Th subset**). Further gating on CCR6, CCR4, CXCR3, and CCR10 distinguishes the remaining Th subsets: Th1 (CXCR5-CCR6-CXCR3+CCR10-), Th2 (CXCR5-CCR6-CXCR3-CCR10-), Th17 (CXCR5-CCR6+CCR4+CXCR3-CCR10-), and Th22 (CXCR5-CCR6+CCR4+CXCR3-CCR10+) [3, 4] (**Figure 1D, Th22_Th17** and **The1_Th2_ThGM**).

Although further subsets of CD8+ T cells are not rigorously defined, high-dimensional analysis with t stochastic neighbor embedding (tSNE) revealed differences in normal human PBMCs according to chemokine and Fc receptor expression (**Figure 1E**). In this example, tSNE discriminated distinct populations of CD8+ Tem cells, which on further examination, segregated according to CCR6, CCR4, and HLADR/CD38 expression. Interestingly, a single prior report has postulated this CD8+ CCR6+ Tem subset as a modulator of mucosal immunity [13], and another report identified CD8+CCR4+ cells as potential mediators of synovial inflammation in rheumatoid arthritis [14]. Thus, this high-color flow panel allows high-dimensional data visualization techniques to uncover unknown and/or poorly-defined cell types in both normal and diseased states.

In summary, our 21-color panel provides a powerful tool for in-depth analysis of lymphoid and myeloid cells in the human peripheral blood with deep T cell analysis and coverage of most populations defined in the NIH's Human Immunology Project. Future panels could substitute certain T cell markers (e.g. CCR4, CXCR5, CCR10) in favor of increased B cell discrimination (e.g. CD19, CD27, IgD). Of note, by comparison to CyTOF, which can simultaneously detect 20–40 antigens, this panel requires fewer input cells, less acquisition time, and less money, while still permitting worthwhile high-dimensional analysis.

Human Subjects

Peripheral blood mononuclear cells were obtained from healthy donors. The use of human tissue in this study was approved by the Institutional Review Board at Washington University in St. Louis.

Similarity to Published OMIPs

This panel builds upon OMIPs −024, −015, and −030, which identify pan-leukocytes, T regulatory cells without intracellular staining, and all major T helper subsets, respectively. This single 21-color panel identifies the majority of subsets described in these three OMIPs, captures the major lymphoid and myeloid immunophenotypes defined in the NIH's Human Immunology Project [3], and uniquely allows for detailed chemokine receptor analysis on non-B/T cell subsets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Mahnke Y , Chattopadhyay P , and Roederer M Publication of optimized multicolor immunofluorescence panels. Cytometry A, 2010 77A(9): p. 814–8.

2. Finak G , et al., Standardizing Flow Cytometry Immunophenotyping Analysis from the Human ImmunoPhenotyping Consortium. Sci Rep, 2016 6: p. 20686.26861911

3. Maecker HT , McCoy JP , and Nussenblatt R , Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol, 2012 12(3): p. 191–200.22343568

4. Wingender G and Kronenberg M , OMIP-030: Characterization of human T cell subsets via surface markers. Cytometry A, 2015 87A(12): p. 1067–9.

5. Cooke KR , et al., The Biology of Chronic Graft-versus-Host Disease: A Task Force Report from the National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease. Biol Blood Marrow Transplant, 2017 23(2): p. 211–234.27713092

6. Zeiser R , Socie G , and Blazar BR , Pathogenesis of acute graft-versus-host disease: from intestinal microbiota alterations to donor T cell activation. Br J Haematol, 2016 175(2): p. 191–207.27619472

7. Michel T , et al., Human CD56bright NK Cells: An Update. J Immunol, 2016 196(7): p. 2923–31.26994304

8. Mahnke YD , Beddall MH , and Roederer M , OMIP-015: human regulatory and activated T-cells without intracellular staining. Cytometry A, 2013 83A(2): p. 179–81.

9. Zhu J , Yamane H , and Paul WE , Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol, 2010 28: p. 445–89.20192806

10. Meditz AL , et al., HLA-DR+ CD38+ CD4+ T lymphocytes have elevated CCR5 expression and produce the majority of R5-tropic HIV-1 RNA in vivo. J Virol, 2011 85(19): p. 10189–200.21813616

11. Seddiki N , et al., Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med, 2006 203(7): p. 1693–700.16818676

12. Liu W , et al., CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med, 2006 203(7): p. 1701–11.16818678

13. Kondo T , Takata H , and Takiguchi M , Functional expression of chemokine receptor CCR6 on human effector memory CD8+ T cells. Eur J Immunol, 2007 37(1): p. 54–65.17171755

14. Cho BA , et al., Characterization of effector memory CD8+ T cells in the synovial fluid of rheumatoid arthritis. J Clin Immunol, 2012 32(4): p. 709–20.22367266

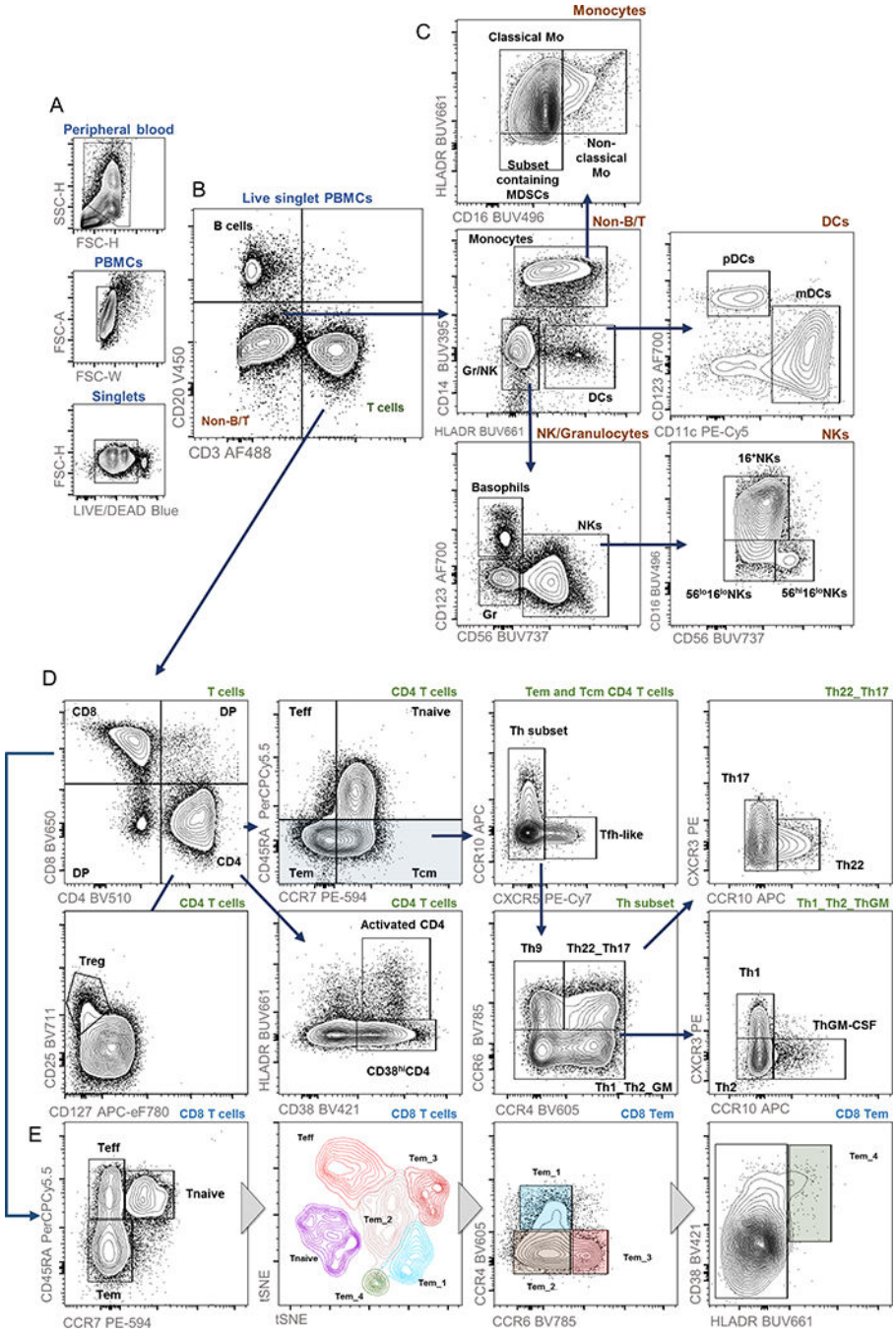

Figure 1.

Example gating strategy for major immune cell subsets on stained PBMCs from healthy donors.

Table 1

Purpose	Myeloid and lymphoid comprehensive immunophenotyping
Cell types	Human PBMCs
Cross-reference	OMIP-030, OMIP-015, OMIP-024

Table 2

Specificity	Fluorochrome	Ab Clone	Purpose
CD14	BUV395	MΦP9	monocytes
Live/Dead	n/a	n/a	viability
CD16	BUV496	3G8	monocytes
HLADR	BUV661	G46–6	DCs
CD56	BUV737	NCAM16.2	NKs
CD38	BV421	HIT2	activation
CD20	BV450	L27	B cells
CD4	BV510	SK3	CD4
CD194/CCR4	BV605	L291H4	Th subset
CD8	BV650	RPA-T8	CD8
CD25	BV711	2A3	Treg
CD196/CCR6	BV785	G034	Th subset
CD3	AF488	UCHT1	T cells
CD45RA	PerCP-Cy5.5	H1100	naïve/memory
CD183/CXCR3	PE	1C6	Th subset
CD197/CCR7	PE-CF594	150503	central/effector
CD11c	PE-Cy5	Bly6	mDCs
CD185/CXCR5	PE-Cy7	RF8B2	Th subset
CCR10	APC	314305	Th subset
CD123	AF700	32703	pDCs
CD127	APC-eF780	RDR5	Treg