Identification of NK Subsets in Human PBMCs via Multi-Parameter Flow Cytometry

OVERVIEW

Flow cytometry is a powerful tool for characterization of immune cells, with applications in immunology, oncology, virology, and other fields. Here, we demonstrated the ability to identify NK cell subsets in human PBMC samples with a simple panel of four surface markers.

BACKGROUND

NK (natural killer) cells are cytotoxic lymphoid cells that are a part of the innate immune system. They are involved in viral defense as well as the elimination of tumorigenic cells, among other roles.[1] Because of their direct tumor-killing abilities, NK cells have become an attractive candidate in cancer immunotherapy research.[2] Consequently, research methods for characterizing NK cells and studying their functionality and modulation have become increasingly important.

The vast majority of human NK cells can be identified by their expression of CD45 and CD56, and their lack of expression of CD3. Although the human immune system possesses a diverse repertoire of NK cell subsets, the major populations in peripheral blood consist of $CD56^{bright}CD16^{-/dim}$ and $CD56^{dim}CD16^{bright}$, with the $CD56^{dim}CD16^{bright}$ population being the most abundant.[3] $CD56^{bright}CD16^{-/dim}$ NK cells are immature cells and/or cytokine producers which are weakly cytotoxic, while $CD56^{dim}CD16^{bright}$ cells are mature, cytotoxic NK cells.[4]

MULTI-PARAMETER FLOW CYTOMETRY METHOD

Cryo-preserved human peripheral blood mononuclear cells (PBMCs) (BioIVT) were thawed in complete medium, rested for 2 hours at 37°C, and subsequently stained for flow cytometry.

The flow cytometry antibody panel included surface markers to identify NK cells, consisting of CD45, CD3, CD56, and CD16. Antibodies were purchased from Cell Signaling Technology (catalog no. 83586S). Single color compensation controls (cell-based) and FMO controls were included for each marker in the panel.

Flow cytometry staining method: PBMCs were stained with a Live/Dead viability stain (Invitrogen) for 30 minutes at 4°C. Cells were washed in PBS and resuspended in the antibody mixture (or control mixture) containing Fc block, and incubated for 1 hour at 4°C. After staining, samples were washed twice and resuspended in staining buffer (PBS + 0.5% BSA). Samples were acquired on the Cytoflex S flow cytometer (Beckman Coulter), and data was analyzed in FlowJo v10.

Table 1. Flow cytometry antibody panel.

Target	Fluorochrome	Vendor, Catalog Number
Live/Dead	Near IR (780)	Invitrogen, L10119
CD45 (HI30)	FITC	CST, 86532
CD3 (UCHT1)	violetFluor 450	CST, 61347
NCAM1 (CD56) (MY31)	APC	CST, 51997
CD16 (3G8)	PE	CST, 82004

FLOW CYTOMETRY GATING STRATEGY & RESULTS

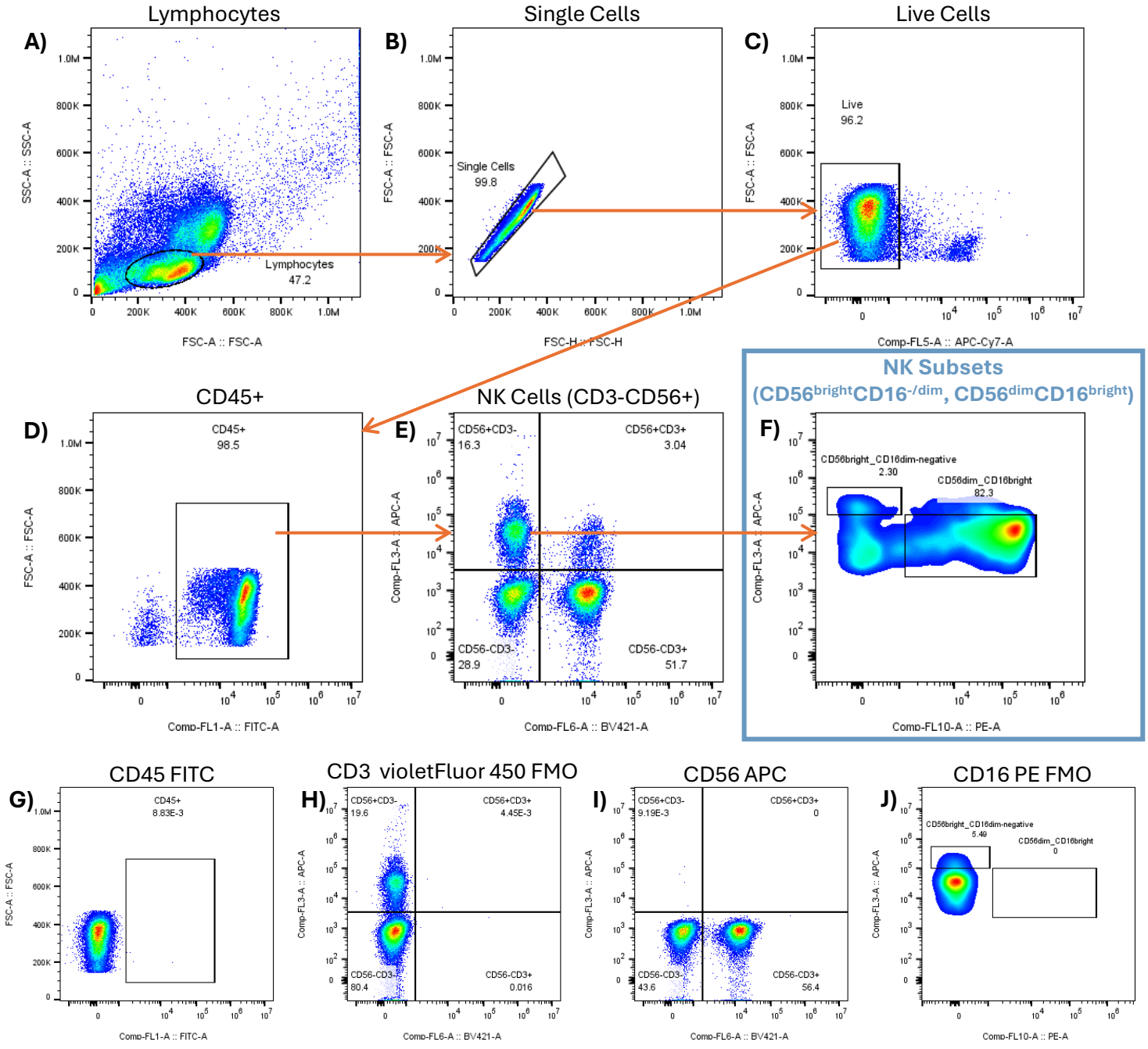

Figure 1. Gating strategy & Identification of NK Subsets. Samples were first gated on the lymphocyte population (A), followed by single cells (B), live cells (C), and CD45+ cells (D). Within the CD45+ population, NK cells were distinguished by CD56 positivity and CD3 negativity (E). CD56+CD3- NK cells were then assessed for relative CD56 and CD16 expression to identify NK subpopulations (F). Positive gates for CD45, CD56, CD3, and CD16 were guided by fluorescence minus one (FMO) control samples (G-J).

SUMMARY

Subsets of circulating NK cells were successfully identified in human PBMC samples using a simple flow cytometry panel of four surface markers.

Flow cytometry is a valuable and versatile method for studying immune cells. Application of the present workflow in future studies will enable the identification of NK cell subsets with a minimal number of antibodies, reserving a larger selection of fluorochrome options for studying treatment effects and downstream targets. Applications include research efforts in immuno-oncology, autoimmune disorders, virology, etc.

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