Application Information Bulletin: Human NK Cells

Phenotypic characterizing of human Natural Killer (NK) cell populations in peripheral blood

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INTRODUCTION

NK cells are part of the innate immune system and implicated in tumor surveillance and killing of virally infected cells. More recently, it has been shown that NK cells also play a role in autoimmune diseases either by regulating the adaptive immune response i.e. T cells or interaction with other regulatory cells, like macrophages, and dendritic cells. We are interested in comparing the NK cell compartment, phenotypically and functionally, of Type 1 diabetic patients to healthy subjects. We are comparing ratios of $CD56^{hi}/CD16^{lo}$ and $CD56^{lo}/CD16+$ NK cells and monitor differential expression of the cell surface receptors including CD8, CD11c, CD38, CD57, and CD117 as well as their expression levels.

SAMPLE PREPARATION

1. Add 35 mL of fresh human blood (received from blood bank) with 20 mL PBS.
2. Add 15 mL of Ficoll-Paque to 50 mL conical tube and transfer of 35 mL PBS diluted blood.
3. Centrifuge tubes at 400 x G for 30 minutes at room temperature without break.
4. Using a (10-25 mL) pipette or a plastic Pasteur pipette gently aspirate the interphase cells from tubes and transfer into new 50 mL conical tubes.
5. Fill the remaining volume (up to 50 mL) with sterile PBS.
6. Wash. Centrifuge tube at 300 X g for 10 min, room temperature (RT), low brake.
7. Lyse blood red cells (BD cat# 555899, 10 X solutions. Use it at 1X in distilled water. Incubation 15 min RT).
8. Resuspend $1x10^6$ PBMCs in 20 µL staining buffer (PBS with 5% Normal Human Serum).
9. Add antibodies: CD117-BV421 (c-kit), CD7-BV510, CD57-BV605, CD8-BV650, CD14-PE, CD19-PE, CD3-PE, CD66b-PE, CD56-PE-Cy7, CD11c-APC, CD45-AF700, CD38-APC-AF750 (all 5 µL per test), and CD16-FITC (20 µL per test) and incubate for 30 minutes on ice.

10. Centrifuge at 300 x G for 5 minutes at 4^0C.
11. Aspirate supernatant.
12. Resuspend in 500 µL staining buffer.
13. Acquire on CytoFLEX[1].

Laser	405nm					488nm					638nm		
Fluor	KrO[2]	PB[3]	V610	V660	V780	FITC	PE	ECD	PC5.5	PC7	APC	APC A700[3]	APC A750[3]
Marker	CD7	c-kit	CD57	CD8		CD16	CD14, CD19, CD3, CD66b			CD56	CD11c	CD45	CD38
Clone	M-T701	104D2	NK-1	SK1		3G8	M5E2 HIB19 UCHT1 G10F5			B159	S-HCL-3	HI30	LS198-4-3

DATA ACQUISITION ON CYTOFLEX

1. Create new compensation.
2. Check each single color control separately and change gain, where applicable.
3. Acquire single color controls (antibody stained VersaComp[2] beads catalog # B22804).
4. Create new experiment.
5. Import previously established compensation settings for BV421, BV510, BV605, BV650, FITC, PE, PE-Cy7, APC, AF700, and APC-AF750.
6. Create following plots: CD45 by SSC, gating on CD45+ cells; FSC by SSC, gating on lymphocytes; FSC-A by FSC-W, gating on single cells; Exclusion by CD7, gating on exclusion-/CD7+ cells; CD16 by CD56, gating on $CD56^{hi}/CD16^{lo}$ and $CD56^{lo}/CD16$+ cells; display for each NK cell subset ($CD56^{hi}/CD16^{lo}$ and $CD56^{lo}/CD16$+) following plots: c-kit by CD38, CD57 by CD11c, and CD57 by CD8.
7. Run the sample on medium.
8. Auto-adjust for scaling.
9. Acquire 250,000-500,000 events.
10. Adjust compensation.
11. Save data.
12. Export to FCS.

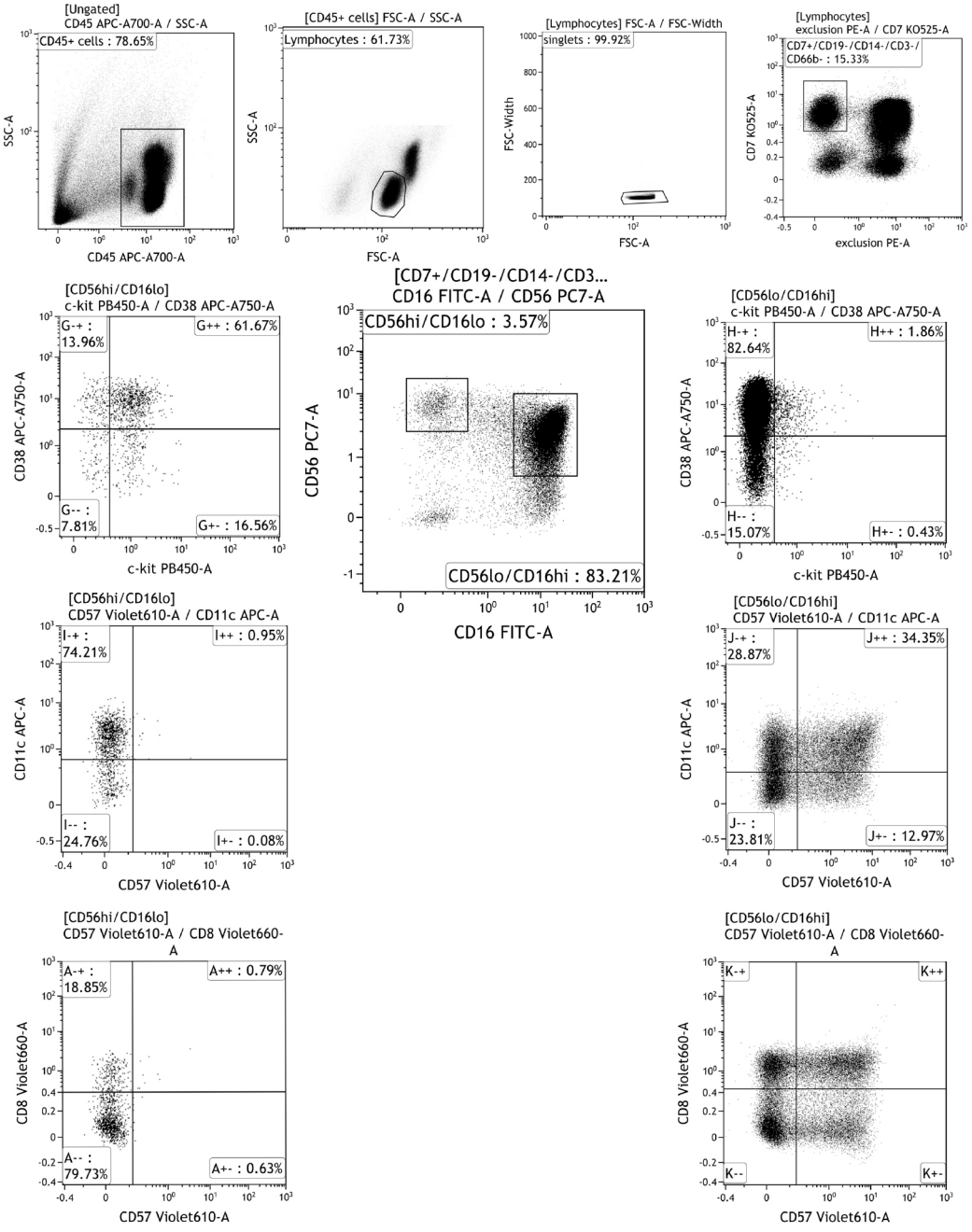

Figure legend:

PBMCs from a healthy donor were stained with CD117-BV421 (c-kit), CD7-BV510, CD57-BV605, CD8-BV650, CD14-PE, CD19-PE, CD3-PE, CD66b-PE, CD56-PE-Cy7, CD11c-APC, CD45-AF700, CD38-APC-AF750, and CD16-FITC. NK cells were defined as CD45+, CD7+, CD19-/CD3-/CD14-/CD66b- (exclusion-). Aggregates were excluded by electronic gating using a FSC-A by FSC-W plot. In humans, two major NK subsets can be identified using the cell surface antigens CD56 and CD16, i.e. $CD56^{hi}/CD16^{lo}$ and $CD56^{lo}/CD16+$. $CD56^{hi}/CD16^{lo}$ and $CD56^{lo}/CD16+$ NK cells were further subdivided based on the expression of CD57, CD11c, CD8, CD38, and CD117 (c-kit).

CONCLUSIONS

NK cells are implicated in autoimmune diseases and may also play a role in T1D progression by creating a regulatory environment that favors the destruction of pancreatic beta cells. The current panel was aimed at identifying NK cell subsets that differ phenotypically between healthy subjects and patients suffering from T1D. The final goal is to establish biomarkers that are predictive in the early, pre-onset phase of T1D.

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