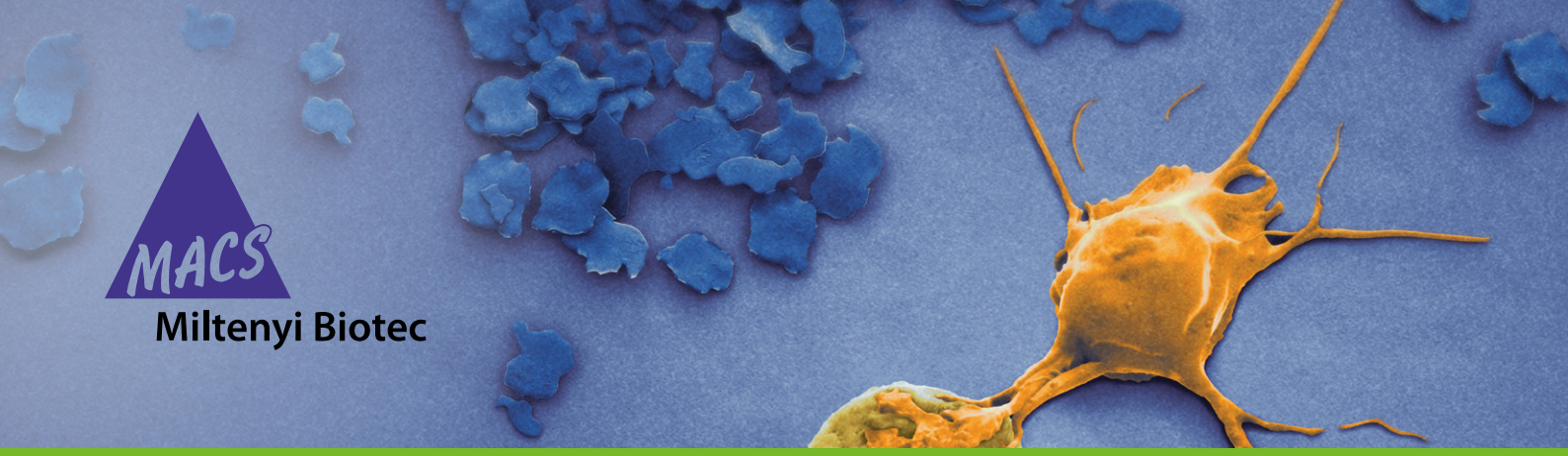

Comprehensive flow cytometry analysis of isolated NK cells from whole blood

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Introduction

Natural killer (NK) cells are part of the innate immune system. They mediate responses against viruses, parasites, bacteria, and tumor cells. Additionally, NK cells contribute to the adaptive immune response by linking innate and adaptive immunity through their receptor, FcγRIIIA (CD16). We describe multicolor flow cytometry analysis necessary to study NK cell phenotype and function of isolated NK cells from whole blood.

Material and methods

Blood samples

Whole blood samples from three donors were collected and used immediately. Untouched NK cells were isolated from 30 mL of human EDTA anticoagulated whole blood using the MACSxpress® Whole Blood NK Cell Isolation Kit, a MACSmix™ Tube Rotator, and a MACSxpress Separator.

Preparation of solutions and staining cocktails

Buffer

PBS/EDTA buffer was supplemented with HSA at a final concentration of 0.5%.

Staining cocktails

Staining cocktails for cell analysis were composed according to panels in table 1. The concentrations of the individual fluorochrome-conjugated antibodies are specified in the corresponding antibody data sheets.

	Panel 1	Panel 2	Panel 3
VioBlue®	CD3	CD3	CD3
APC-Vio®770	CD56	CD56	CD56
APC	CD16	NKp44	NKG2C
FITC	KIR2D	CD69	KIR2D
PE	NKG2C	CD25	
PE-Vio615	NKG2D	NKp46	CD57
PE-Vio770	NKG2A	NKp30	CD107a

Table 1: Composition of staining cocktails.

Cell staining

Cells were resuspended in 110 µL of the respective staining cocktail, protected from light and incubated for 10 min at 2–8 °C. After antibody incubation, cells were washed and resuspended in 500 µL of PBS/EDTA buffer for analysis with the MACSQuant® Analyzer 10 and Flowlogic™.

Gating strategy

Cells were analyzed by flow cytometry using the MACSQuant® Analyzer 10. Cell debris was excluded from analysis based upon scatter signals. Subsequently, doublets were excluded and NK cells were identified as $CD56^{+}$ $CD3^{-}$. Further $CD56^{bright}$ and $CD56^{dim}$ subsets were identified using CD56 vs. CD16 (figure 1).

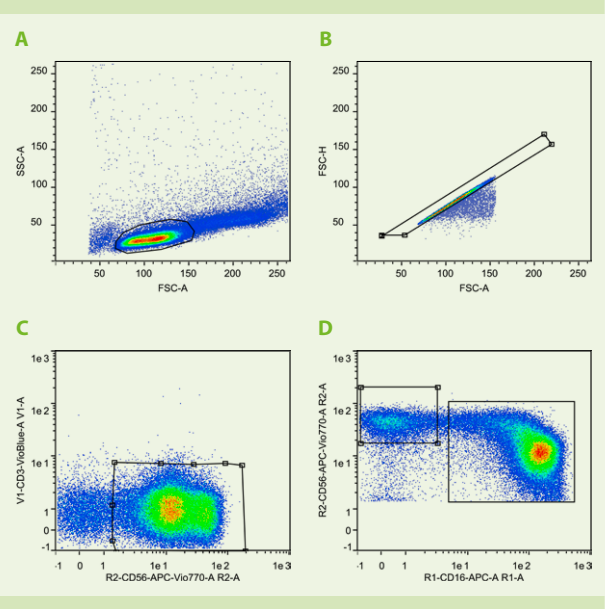

Figure 1: Gating strategy. NK cells are defined as $CD3^{-}CD56^{+}$ (C). Lymphocytes were included and dead cells were excluded using gate A and doublets were excluded from the analysis using gate B. NK cell subsets are defined as $CD56^{bright}$ ($CD56^{bright}$ $CD16^{-}$) and $CD56^{dim}$ ($CD56^{dim}$ $CD16^{+}$) (D).

Results

To mediate adequate protection, NK cells need to distinguish between normal and tumor or virus infected cells. Killing of specific targets is based on the interaction with target cells via a complex system of germ-line coded activating and inhibitory receptors. The group of NK cell receptors signaling through an immunoreceptor tyrosine-based activating motif (ITAM) consisting of activating KIRs, NKG2C, natural cytotoxicity receptors (NCRs) and CD16. Some common surface activation markers include NKp44, CD69 and CD25. Once NK cells release cytotoxic granules, the degranulation marker CD107a (LAMP-1) can also be found on the cell surface. NK cells without stimuli are negative to these markers, as observed in figure 2 (representative donor), NK cells from a healthy donor after isolation using MACSxpress® Whole Blood NK Cell Isolation Kit.

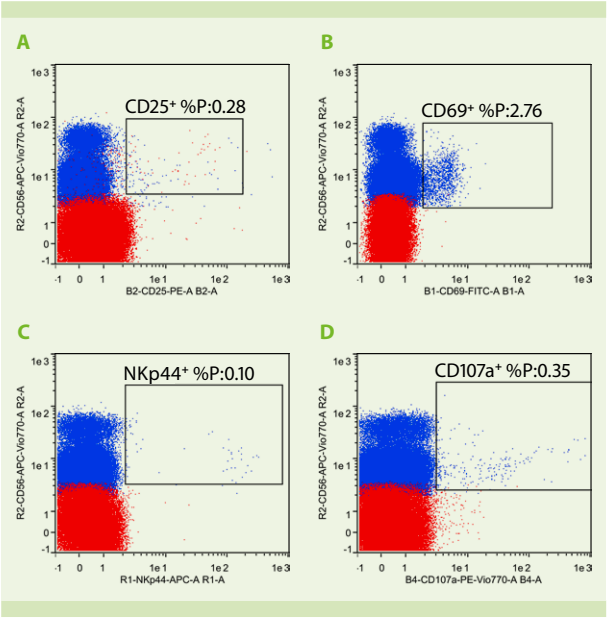

Figure 2: NK cell activation markers after isolation. NK cells ($CD3^-CD56^+$) were stained with CD25 (A), CD69 (B), NKp44 (C) and CD107a (D) after isolation with MACSxpress® Whole Blood NK Cell Isolation Kit. Overlay with isotype controls (red).

The $CD56^{bright}$ and $CD56^{dim}$ subsets have different roles and express different cell surface repertoire (figure 3). Panel 1 allows the detection of NKG2 receptors in $CD56^{bright}$ and $CD56^{dim}$ subsets as well as $KIR2D^+$ cells. Additionally NK cells can be analyzed for their activating receptors; for instance expression using NKp30, and NKp46 (figure 4).

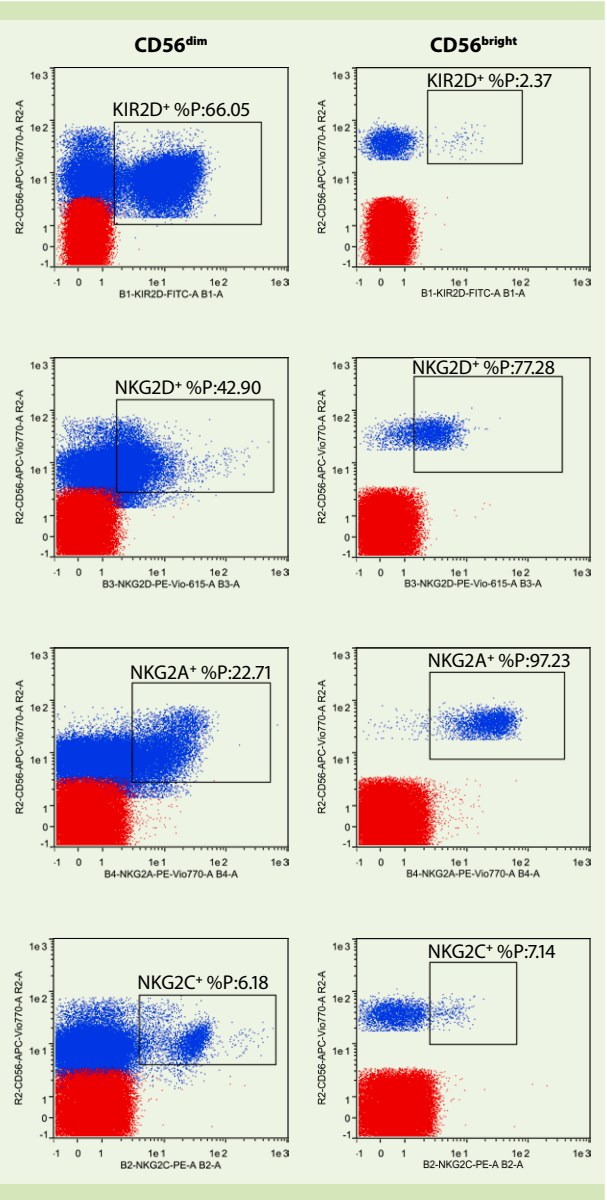

Figure 3: NK cell subsets and expression of KIR2D and NKG2 receptors. NK cell subsets were analyzed for their expression of KIR2D, NKG2D, NKG2A and NKG2C. Left column depicts $CD56^{dim}$ subset and right column $CD56^{bright}$ subset. Overlay with isotype controls (red).

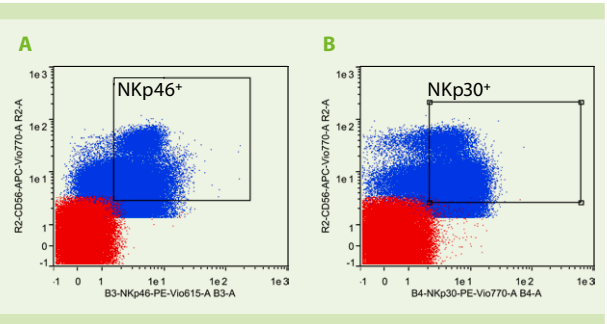

Figure 4: NK cell expression of activating receptors. NK cells ($CD3^-CD56^+$) were analyzed for their expression of activating receptors. Figure depicts $CD3^-CD56^+$ NK cells gated on NKp46 (A) and NKp30 (B). Overlay with isotype controls (red).

Finally, $CD57^+$ NK cells have been described to have a more mature phenotype, and combination with other markers like NKG2C are characteristic of adaptive or memory-like NK cells (figure 5). This subset of $NKG2C^+$ NK cells upregulates CD57 and uniquely expresses self-HLA-C-reactive KIR receptors.

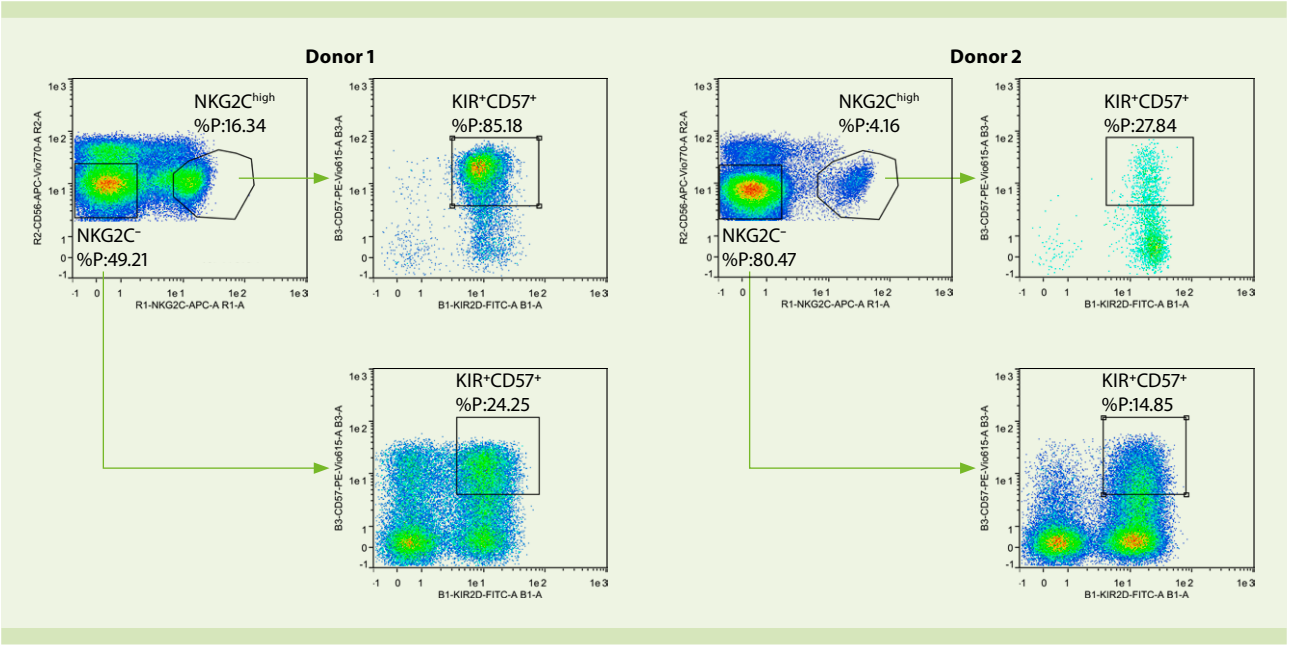

Figure 5: Adaptive or memory-like NK cells. NK cells from two donors ($CD3^-CD56^+$) were analyzed for their expression of NKG2C. Subsequent gating on $NKG2C^{high}$ and $NKG2C^-$ subsets shows the presence of KIR^+CD57^+ adaptive or memory-like NK cells.

Conclusion

- Presented data confirms the feasibility of fast NK cell isolation and evaluation of the presence of NK cell receptors.
- Flow cytometry analysis was based on three different antibody panels.
- REAfinity™ antibodies allow the of functional and phenotypic study of NK cells.

References

Reagents	Order no.
Red Blood Cell Lysis Solution (10×)	130-094-183
MACS Comp Bead Kit, anti-REA	130-104-693

Instruments and software
MACSQuant Analyzer 10
MACSQuantify Software

Antibodies	Fluorochrome**	Clone
CD107a	PE-Vio770	REA792
CD16	APC	REA423
CD25	PE	REA945
CD3	VioBlue	REA613
CD56	APC-Vio770	REA196
CD57	PE-Vio615	REA769
CD69	FITC	REA824
KIR2D	FITC	REA1042
NKG2A	PE-Vio770	REA110
NKG2C	PE	REA205
NKG2C	APC	REA205
NKG2D	PE-Vio615	REA797
NKp30	PE-Vio770	REA823
NKp44*	APC	2.29*
NKp46	PE-Vio615	REA808

*Now available REA1163

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